THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

VOL. LXIII—No. 2

Issued January 1, 1923

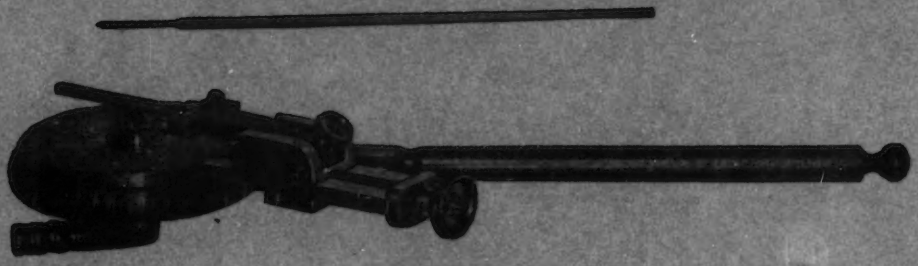
BALTIMORE, U. S. A.

1923

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 5, 1918.

Made in United States of America

BECKER AIR TAMBOUR



THE instrument pictured above is very sensitive to changes in air pressure, but is so well made that it will give satisfactory service for years. The extent of the excursion of the writing point can be increased or decreased by turning a milled screw head. It can be adjusted to any position in the horizontal plane and its level is adjusted by means of a small screw. The entire surface of the rubber, except for a small central area, is protected by a metal diaphragm. The rubber is laid on the air capsule, a brass ring is placed over it, and then the air capsule is screwed against the metal diaphragm. In this way tying or stretching of the rubber is eliminated.

WHEN not in use, a small cap is screwed over the central area, and if a cork is pushed into the air-tube the capsule is rendered air-tight and the rubber dam will last indefinitely.

PRICE $14.50

Catalogue on Request

JOSEPH BECKER, College of Physicians and Surgeons, 437 West 59th Street New York

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 63 JANUARY 1, 1923 No. 2

THE RETINAL REFLEX IN FROGS

V. H. K. MOORHOUSE

From the Department of Physiology and Pharmacology, Medical College, University of Manitoba, Winnipeg, Canada

Received for publication October 9, 1922

The muscular response to changes of illumination produced in frogs under the influence of benzene has been described by Cameron and Sedziak (1). Fröhlich and Kreidl (2) independently observed the light reaction in prawns (Palaemon), under the influence of camphor and phenol. The fact that this retinal reflex is a very general phenomenon in free moving animals has been brought out by Cameron and O'Donoghue (3), who have examined many species of sea animals, using benzene, phenol and camphor as narcotizing agents. It is interesting in this connection to recall Garrey's (4) work demonstrating the undoubted influence of light on the muscle tonus of insects. This author has given us an example of a continuous influence set up by the action of light on the retina and exerted on muscle tone. In the animals here studied the retinal receptors elaborate an impulse resulting in some rapid muscular movement of a protective nature. In the normal animal living under natural conditions this reaction to light and shadow is evidently an important protective reflex. Under natural conditions the immediate diving of frogs in a pool when a shadow falls is a well-known response to a change of illumination. When studied in the laboratory normal frogs show little or no indication of a response to light changes. Benzene and phenol in proper dosage render the muscular response inevitable. It therefore becomes a question of interest to investigate what parts of the central nervous system are concerned in intermediating the reflex. The question also arises as to whether these drugs act by relieving the path of some inhibitory influence by narcotization or by

increasing the irritability (sensitization) of some element in the path of the reflex. These questions have been made the subject of the present investigation.

METHODS. Frogs (R. pipiens, Illinois) as large as available were used in the experiments. Under very light ether anesthesia or with no anesthesia, the fronto-parietal bones were removed with fine scissors, and various portions of the brain were either removed with fine forceps and curettes or cauterized with a heated needle. After being operated upon the animals were left in their aquarium for a day or more to ensure full recovery from shock or anesthesia.

Their behavior on recovery was carefully observed, and the effect of the drug used (in most cases benzene) on the incidence of the response to changes of illumination studied.

The dosage of benzene used was in all cases 1 per cent of the body weight of the pure compound, which has been found by Cameron and Sedziak (1) to be the optimum dose.

Normal frogs as controls. Parallel injections of normal frogs were carried out in every observation on the effects of removal of different parts of the nervous system. This is necessary in order to compare the effects under like conditions of temperature, lighting, etc.

Incidence of the light response. It will be seen in table 1 that the light response occurred in 31 intact frogs out of a total of 32 experiments. The average time of appearance of the light response in the uninjured frogs was 27 minutes after injection of benzene. An ordinary shaded 60 watt Tungsten lamp was used as the source of light, care being taken that the switch operated without noise. The light "on" stimulus in this paper refers to turning the lamp on and is therefore a sudden change in the direction of greater brightness; the light "off" signifies turning off the lamp and is therefore a change toward darkness. The type of response when first seen is a slight start of the animal as it sits in a crouched, head-low position. Usually the light "on" stimulus is first effective. When the reflex becomes fully developed the response to light "off" is more marked and amounts to an initial "backwater" movement (abduction of the legs) quickly followed by a violent extension of the hind legs with minor movements of the head and fore limbs. When placed in a deep water bath the resultant movement of the animal is first to steady the body and then to dive. Under the influence of the narcotics the purpose of the movement is not very effectually carried out, but the muscular response is usually very violent, the animal frequently splashing water out of the bath. It will be noted that with

intact frogs and using the proper dosage of benzene the response is practically inevitable. With smaller doses the light response may not appear. With larger doses the animals frequently go quickly into a state of rigor and do not respond to light changes or other stimuli.

Using a 0.1 per cent solution of phenol in Ringer's fluid and administering 0.5 cc. per each 10 grams of weight of the normal frog, none of the marked narcotic effects are seen as with benzene. The frog retains its normal posture and moves round actively. The light response will be found to appear in from 10 to 15 minutes and to last for variable periods up to 3 hours. Complete recovery from such a dose is usual and these frogs have been used several times for such experiments. With larger doses of phenol recovery is not likely to occur. The type of response to changes of illumination is essentially the same as that observed with benzene, the frog showing, however, none of the depression which ensues after an adequate dose of the latter substance.

Both benzene and phenol also increase the excitability of the frog to mechanical stimuli of a vibrating nature. A slight tap on the table or dish in which the animal is placed is sufficient to produce a quick contraction of all body muscles. With injury to the optic lobes this response is very active, although it is then impossible to obtain any response to change of illumination. This response is referred to here as "jar."

EXPERIMENTS IN SECTION AND REMOVAL: *1. Section and removal of the eyes.* Removal of both globes of the eyes abolishes the reflex. Removal of one eyeball still permits the response to appear. Removal of the lens and iris on both sides does not prevent the incidence of the light response.

2. Median section of the brain. A median division of brain with the object of cutting the optic commissure (fig. 1). In 5 experiments (see table 1) no light response was elicited after this section. This confirms what has been stated before that there is present a total crossing of fibers in the optic commissure of the frog. (See Gaupp, p. 87 (5).)

3. Removal of cerebral hemispheres (fig. 1 *a*). In table 1 it will be seen that in 16 such removals the light response was present after benzene in 13 cases and absent in 3 instances. Amongst the latter it was noticeable that responses to other forms of stimulation were wanting. The average time of appearance of the first light response after injection of the drug was 16.0 minutes. The removal of the cerebral hemispheres, therefore, does not interfere with the development of the reflex. In fact the time taken for the response to appear was, on the whole, shorter,

and in a number of instances there appeared to be a much stronger response. The reason for this may be that the drug is more effective in the slightly impaired condition of the animal following removal of brain tissue. It should be mentioned that no indication of a light response has been seen in frogs with the cerebral hemispheres removed, in which benzene or phenol has not been injected.

4. Removal of and injury to the optic tectum. When the entire optic lobes, figure 1, *d*, *e*, on both sides were removed (in some cases this

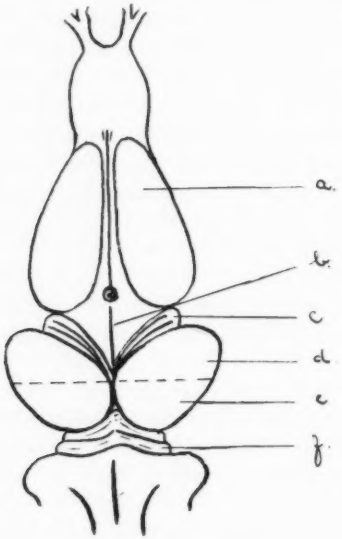


Fig. 1

was done by actual cautery, but removal is best accomplished with fine scissors) the light response failed to appear. Out of 14 such removals the light response appeared in 3 cases only (table 1) after the injection of benzene. In all these cases of non-appearance of the light response, other responses—such as the "jar" reflex—were very active and readily elicited.

In four experiments the anterior portion of the tectum opticum (fig. 1, *d*), was carefully cauterized. The light response was elicited in only one of these. The light response was fully absent in the cases of three, while their sensitivity to other forms of stimulation was marked.

When the posterior portion of the tectum opticum (fig. 1, *e*), is removed or cauterized on both sides, the light response was not always interfered with. In 8 experiments the reflex appeared in 6 cases and was absent in the other two; average time, 20 minutes.

The optic tectum is obviously shown by these experiments to be an important intermediary station in the path of the impulses set up by light stimuli. The anterior portion of the tectum opticum appears to have the greater importance than the other parts, as, when injured, the response to light almost always fails to appear.

TABLE I

Summary of retinal reflex experiments

PARTICULARS	NUMBER OF EXPERIMENTS	RETINAL REFLEX		OTHER[1] RESPONSES	TIME[2] IN MINUTES
		Present	Absent		
Normal	32	31	1	x	27.8
Removed cerebral hemispheres	16	13	3	x	16.0
Optical lobes removed	14	3	11	xx	
Optic tectum anterior part removed	4	1	3	xx	25.0
Optic tectum posterior part removed	8	6	2	xx	20.5
Pars. intercal. dienc. injured	4	3	1	x	19.0
Mid-line division	5	0	5	x	
Lens and iris resected	1	1	0		20.0

[1] "Other responses" refers to the animal's sensitivity to mechanical stimuli such as the "jar." The active response is denoted by an "x" sign and hyperactivity by two "x" signs.

[2] Time refers to minutes elapsing after injection of benzene and before the first appearance of retinal reflex. Average of all experiments.

5. Pars intercal. diencephali (fig. 1, *b*). Removal or cautery of this portion of the brain in 4 cases resulted in the light response failing to appear in one instance only. If precautions are taken not to injure the optic tracts which sweep up from below toward the optic tectum on both sides, extensive injury may be done to this portion of the brain without abolishing the light response.

The distribution of the fibers of the optic tracts and the connections made with the mid-brain are of interest in relation to the path of this light impulse and may be briefly described here. For greater detail the reader is referred to the articles of Gaupp (5), Herrick (6) and Kappers and Hammer (7). The fibers of the optic tracts fall into three

main divisions, the axial, basal and marginal bundles (Wlassak, quoted by Herrick (5)). Of these the basal bundle passes caudad and ends in the cerebral peduncle in the region of the oculomotor nucleus. The marginal bundle, containing the largest part of the peripheral optic fibers, passes superficially caudad and dorsad and enters the tectum in the mid dorsal region. In Necturus it is distributed to the anterior portions of the tectum and does not extend to the caudal end of the tectum (6). The efferent path from the mid-brain to the neurons at lower levels is probably by the ventral tectobulbar fibers which arise from the whole of the optic tectum especially the middle and posterior portion (7) and course backwards. The fact that injuries to the anterior half of the optic tectum abolish the response to light may indicate that here a cell station, which passes the impulse on, is destroyed or that the fibers of the marginal bundle which are here very superficial have been injured.

The observation of Kohlrausch and Schiff (8) that there is a galvanic action current from the skin of frogs subjected to sensory stimuli (including light) suggests that any change of illumination sets in train an impulse which is quite generalized. Having obtained no very marked evidence in the frog of an inhibitory influence exerted by the portions of the brain studied (unless the shortening of the time of development of the reflex in decerebrated frogs under benzene may be taken as such), it occurred that the inevitability of the reaction when benzene and similar compounds are used might depend on the increased irritability of motor cells in the spinal cord. Baglioni (9) in an analysis of the different elements of the spinal cord describes in detail the effect of phenol on frogs. The frog in a short period of time after subcutaneous injection of 0.5 cc. 2 per cent phenol enters into a long-continued stage in which clonic twitchings occur frequently "with slight or no appreciable stimulus." Any movements elicited reflexly are broken by clonic contractions. It is in this stage in our experiments that the light response may be elicited markedly, and if smaller doses of phenol are used, as in our experiments, the light reflex may appear unbroken by the series of clonic convulsions.

Baglioni found that if the solution of phenol were applied to the bared spinal cord in the lumbar region, there quickly ensued a state of anesthesia due to the action of phenol on the posterior roots and no reflexes could be elicited from the hinder areas. Clonic twitchings in the hind limbs constantly appeared, evidently due to impulses coming through from other areas, as section of the cord abolished these twitching movements.

When local applications of benzene and phenol are made to the uncovered spinal cord, the results are exactly as described by Baglioni. Benzene applications appear to spread more rapidly than phenol, and in time the activities of all portions of the spinal segment treated disappear. With a careful local application, it frequently happens that the response to light "on" and "off" involves only one hind limb at first, soon causing twitches of both hind legs, and finally involving the whole of the body muscles in the characteristic light response. One detailed protocol may be given as an example of the ten experiments of this kind done:

July 17, 1922. *Frog 1.* 53 grams. Leopard frog.

2:55. Uncovered spinal cord in lower part—about 4 mm. length exposed. No anesthetic used. Reflexes active and normal.

3:25. Sensation normal in exposed area. Responds normally to pin prick and pinch. No light response.

3:26. Exposed area of cord sponged carefully. No bleeding. Applied small cotton swab moistened with benzene. Sensation of hind limbs quickly lost. Prick and pinch to hind limbs ineffective. Motor power in exposed area good. Responds with movements of hind limbs to reflexes elicited in anterior parts.

3:31. Unmistakable response to light "off" in muscles of right leg only. No response to pinching or pricking hind legs. Fore limbs take no part in light response. Response to "jar" stimuli from hind leg.

3:40. Response to light from both hind limbs, but muscle tone is greatly lessened.

4:05. Light response "on," "off," from the fore limbs only. The hind limbs are flaccid and no movement of them is seen.

With phenol solutions 0.1 per cent locally applied to the exposed cord the results are similar but not so quickly produced as with benzene. With camphor injected or locally applied to the spinal cord in frogs the light response has not so far been elicited.

It therefore seems clear that benzene and phenol bring out the retinal reflex by producing an increased excitability of the anterior (motor) cells of the spinal cord. This increase of motor excitability is also evidenced by the clonic twitchings of muscles and by the ready response to direct mechanical stimuli of a vibrant nature such as the "jar."

The effect of strychnine on the light response has been investigated. Strychnine does not of itself bring out the retinal reflex. Doses just sufficient to cause tetani and given before the light response appears under benzene, do not produce any marked shortening of the time of appearance of the reflex. Strychnine administered after the light

response is established, produces a short lasting period of increase of response followed by tetani. When the frog develops typical strychnine convulsions it is impossible to state whether the light stimulus is effective or not. The type of muscular response to light is changed under strychnine from the quick extension followed by flexion to the extensor tetanus after which the reflex is indistinguishable.

SUMMARY

1. An investigation of the parts of the central nervous system involved in transmitting the "retinal reflex" impulse is here reported.

2. Removal of the cerebral hemispheres appears to shorten the average time appearance of the retinal reflex, and to make the response more active.

3. The area of the frog's brain which is most important in forwarding the impulse is the optic tectum, and especially the anterior portion of its structure.

4. The reason that the outward manifestation (muscular response) of this retinal reflex is made plain under the influence of benzene and phenol lies in the increased excitability of motor cells which is produced under the influence of these substances.

BIBLIOGRAPHY

(1) Cameron and Sedziak: Proc. Physiol. Soc., Journ. Physiol., 1921, liv, xcv.
(2) Fröhlich and Kreidl: Arch. f. d. gesammt. Physiol., 1921, clxxxvii, 102.
(3) Cameron and O'Donoghue: Biol. Bull., 1922, xlii, 217.
(4) Garrey: Journ. Gen. Physiol., 1918, I, 101.
(5) Gaupp: Anatomie des Frosches, 1896.
(6) Herrick: Journ. Compar. Neurol., 1917, xxviii, 216.
(7) Kappers and Hammer: Psychiatrische and Neurologische Bladen Festbundel Winkler, 1918.
(8) Kohlrausch and Schiff: Arch. f. d. gesammt. Physiol., 1922, cxciv, 326.
(9) Baglioni: Arch. (Anat. U.) Physiol., 1900, Suppl. Bd. 193. Zeitschrf. Allg. Physiol., 1909, ix, 24.

AN EXAMINATION OF CERTAIN PROPOSED TESTS FOR FATIGUE

FREDERIC S. LEE AND J. D. VANBUSKIRK

From the Department of Physiology, Columbia University, New York

Received for publication October 10, 1922

With the increased study of fatigue in human beings which has occurred in recent years, the need of suitable tests for fatigue has become increasingly clear. Such tests might be either subjective or objective, and, if objective, might be either physical or chemical. An ideal test would be not only qualitative but quantitative as well, and would thus be a measure of the degree of fatigue of the individual. Whether such an ideal test is possible is here not to be discussed. The inquirer into this problem might read with profit Muscio's recent paper (1).

Several objective physical tests for bodily condition have been devised in recent years, and it is the purpose of the present paper to consider some of these in the light of experimental evidence which we have accumulated. Not all of them have been put forward by their authors primarily as tests for fatigue; but, since fatigue is often an element in bodily condition, they might be assumed to be capable of revealing the presence of this element. Where such an assumption has been made it is not usually made clear by its author whether such fatigue as can be thus detected is that which results from the performance of a specific brief task, such as a day's labor, or the cumulative fatigue of days, weeks or months of overexertion. It is obvious that a test might reveal the latter and yet be useless as an indicator of the former condition. With most of the tests to be discussed in the present paper the experimental evidence that has yet been put forward in favor of their applicability in the detection of daily fatigue is slight; yet it goes without saying that in this field there is just now the greatest need and the greatest demand.

We have used four men, one professor and three undergraduate students, as the subjects of our observations and the latter have been made over a period between April 7 and May 19, 1922, covering a maximum of ninety-four days for the individual. With the thought of the possible

future application of the tests in industry, observations were made twice daily: first, during the forenoon, before the subject had begun his daily task, and again late in the afternoon. Between the morning and the afternoon observations, on certain days, to be called "rest days," the subjects performed only slight physical exercise but spent their time chiefly sitting in the laboratory and studying; on other days, to be called "work days," they walked over a certain course in the city streets, the distance varying between 6 and 16.75 miles but averaging 14 miles daily. For men unaccustomed, as they were, to physical exercise this was a distinctly fatiguing task. In the midst of their walk they obtained luncheon at a restaurant. We shall refer hereafter to the subjects as A, B, C and D, and their respective ages were 40, 23, 19 and 25 years.

Our observations relate to certain cardiovascular, respiratory and muscular strength phenomena, records of which were made in the order named. The morning observations were not begun until at least one and one-half hours had elapsed after the conclusion of the subject's breakfast; the afternoon observations followed a period of three or four hours after the conclusion of his luncheon, but before the evening meal. We thus avoided the influence of the ingestion of food which, Weysse and Lutz (2) have shown, causes a pronounced increase in pulse rate, systolic pressure, pulse pressure and the relative velocity of the circulating blood, immediately following breakfast, luncheon and dinner. On the afternoons of the work days the subjects sat quietly in the laboratory for a period of approximately one hour after the completion of their walk, and the tests were then begun. A period of about twenty-five minutes was required for the performance of all the tests on a single individual. The results will now be presented.

I. Cardiovascular tests. Various cardiovascular phenomena have been believed to reveal evidence of bodily fatigue. Our observations cover the rate of the heart beat in the reclining and the standing bodily positions, the increase in rate on passing from one to the other, the immediate increase in rate after a given exercise, the time required after the given exercise for a return to the original rate, the systolic and diastolic blood pressures in the reclining and the standing positions, the decrease in systolic pressure on passing from one position to the other, the pulse pressure in the two positions, the combination of these various data constituting the Crampton and the Schneider tests, the relative velocity of the circulating blood in both the reclining and the standing positions, the energy of the heart, the peripheral resistance to the circulation of the blood, and the vascular skin reaction.

1. Order of procedure. During each period of observation, whether morning or afternoon, the cardiovascular phenomena were the first to be examined. The subject, who had been sitting in the laboratory for not less than a half hour, lay quietly on his back at full length for a period of five minutes, during which a sphygmomanometric cuff and a bracelet stethoscope were attached to his arm. Continuing in this reclining position his pulse was counted for 20-second periods until constant, and the figure was multiplied by three and recorded. The systolic and diastolic blood pressures were observed by the auscultatory method and recorded. We found frequently that the blood pressures varied according to respiratory phases. Hence in most cases the readings were made during expiration. The subject then arose quietly and remained standing on his feet for two minutes before his pulse and blood pressures were similarly observed in the standing position. Schneider's exercise test was then applied as follows: At the word "ready" the subject placed one foot on the top of a stool, 18 inches in height; retaining this foot in this position, at the successive commands "one, down," "two, down," etc., he alternately raised his body to a standing position on the stool and lowered it to the floor five times in fifteen seconds, and then immediately resumed his standing position on the floor; after five seconds his pulse was counted for twenty seconds and then during fifteen-second intervals until it had resumed its original standing rate. The rate per minute immediately after exercise and the time in seconds required for recovery were recorded. A period of about ten minutes was usually required for making these observations, after which the subject proceeded to the respiratory tests.

2. The heart rate. The data are summarized in table 1.

This table presents few striking features. Comparing the mornings and afternoons of the rest days we find a minute diminution of the heart rate in both bodily positions and a constancy in the increased rate on standing, while after exercise there occur the three features of a minute decrease in the immediate rate, a slightly augmented increase on standing, and an increase of five seconds in the time of recovery. But the reduced rate without exercise occurs, in the reclining position, in but 52 per cent, and in the standing position in but 48 per cent, of the total days; the constancy of the increase on standing occurs in but 22 per cent of the days; while after exercise the decrease in the immediate rate characterizes only 46 per cent, the augmented increase on standing only 47 per cent, and the increase in the time of recovery only 35 per cent, of the total days.

TABLE I

Heart rate

Arithmetic means of all observations expressed in terms of nearest whole number

PERIOD	NUMBER OF OBSERVATIONS	RECLINING		STANDING		INCREASE IN RATE ON STANDING		AFTER EXERCISE					
										Increase over original standing rate		Time of recovery	
		Rate	Number and percentage of days and character of effect	Rate	Number and percentage of days and character of effect	Amount	Number and percentage of days and character of effect	Rate	Number and percentage of days and character of effect	Amount	Number and percentage of days and character of effect	Seconds	Number and percentage of days and character of effect
Rest days a.m.....	64	67		83		16		92		9		37	
Rest days p.m.....	64	65	33 (52%) Diminished	81	31 (48%) Diminished	16	27 (42%) Diminished	91	29 (46%) Diminished	10	30 (47%) Increased	42	27 (42%) Unchanged
			24 (37%) Increased		19 (30%) Increased		23 (36%) Increased		20 (31%) Increased		23 (36%) Diminished		22 (35%) Increased
			7 (11%) Unchanged		14 (22%) Unchanged		14 (22%) Unchanged		15 (23%) Unchanged		11 (17%) Unchanged		15 (23%) Diminished
Work days a.m....	29	68		85		17		96		11		52	
Work days p.m....	29	71	18 (62%) Increased	95	24 (83%) Increased	24	20 (69%) Increased	106	22 (76%) Increased	11	12 (41%) Increased	61	12 (41%) Increased
			6 (20%) Unchanged		4 (13%) Diminished		6 (20%) Diminished		4 (13%) Diminished		12 (41%) Diminished		11 (38%) Diminished
			5 (18%) Diminished		1 (4%) Unchanged		3 (11%) Unchanged		3 (10%) Unchanged		5 (18%) Unchanged		6 (20%) Unchanged

During the work days there occur an increase in the rate of beat, which is more marked in the standing position, an augmented increase on standing, and, after exercise, an increase in the immediate rate and a constancy of the increase over the original standing rate, while the increase in the time of recovery amounts to nine seconds. But here again there is uncertainty, for, while 83 per cent of the work afternoons show a higher pulse rate on standing and 69 per cent a greater increase over the reclining rate, when one compares these percentages with 30 and 36 for the rest days, it is clearly seen that they are not reliable indices of fatigue for individuals or small groups. The same conclusion is even more evident in the case of the increase in the reclining pulse rate, the rate after exercise, and the time of recovery after exercise; the percentages of occurrence here being only 62, 76 and 41, as compared with 37, 31 and 35 for the rest days. One would hardly care to lay much stress on these differences from the standpoint of fatigue.

A survey of the deviations shown in the original records is not more reassuring. In the reclining position the average increase in the heart rate of the work afternoons over that of the rest afternoons, namely 6, is approximately the same as the standard deviation of the rate for the rest afternoons. In the standing position the increased rate of 14 on the work afternoons as compared with the rest afternoons is nearly twice as great as the standard deviation. But even on rest days the two men who furnished us most of the work data exhibited great variations in the standing heart rate; one showed a standard deviation of 7.9 with extreme deviations from the mean of $+10$ and -17, the other a standard deviation of 8.2 with extremes of $+13$ and -20. The variation on work days is about the same, so that, while the averages show what might be considered a fatigue effect, the results are so inconstant as to make this a doubtful criterion of fatigue in such cases as we have studied. After exercise also the variations in the original results are too great to justify the use of the feature as a test for fatigue.

We have also examined the figures of heart rates for the mornings following the work days in order to discover possible evidences of fatigue, but we have found no features here to arrest the attention.

We are thus forced to renounce the pulse rate as an index, by itself, of the physical fatigue resulting from the day's work.

3. The blood pressure. The data are summarized in table 2.

There appears to be nothing here that contributes to our knowledge of fatigue. The slight changes in the pressures from morning to afternoon on the work days were found to be approximately the same as the

calculated standard deviations. The lessened pulse pressure in the standing position on the work days was found to occur in only 76 per cent of the days; on the other days the pulse pressure was either increased or unchanged. Evidently blood pressure data alone cannot be used as a criterion of daily physical fatigue.

4. The Crampton and the Schneider tests. In 1905, and again in 1913, Crampton (3) proposed a test for physical condition based on the change of the heart rate and the systolic blood pressure in passing from the reclining to the standing position. The results were expressed in the form of a single figure, which was taken from a scale of percentages constructed from a wide range of variations in the two phenomena. A mark of 100 indicated a perfectly working cardiovascular system, one of zero a seriously inefficient system. The method has been found use-

TABLE 2

Blood pressure

Arithmetic means of all observations expressed in terms of nearest whole number

PERIOD	NUMBER OF OBSERVATIONS	RECLINING			STANDING			DECREASE in SYSTOLIC PRESSURE on STANDING
		Systolic	Diastolic	Pulse pressure	Systolic	Diastolic	Pulse pressure	
Rest days a.m.	64	116	76	40	112	81	31	4
Rest days p.m.	64	116	78	38	113	83	31	3
Work days a.m.	29	118	76	43	113	79	35	5
Work days p.m.	29	116	75	41	110	80	30	6

ful by its author and others in detecting physical deterioration and unfitness. The New York State Commission on Ventilation (4) observed that the index was lowered by exposure of the individual to a hot and humid atmosphere.

In 1920 Schneider (5) devised a test for physical fatigue and efficiency based on six data: The reclining pulse rate, the standing pulse rate, the change in the pulse rate and in the systolic pressure on standing, the increase of the standing pulse rate immediately after a standard exercise (described on page 187), and the time required for the return of the pulse after the exercise to the original standing rate. According to an arbitrary system of rating these data the highest possible score which the individual could obtain was 18, and a subject having a score of 9 or less was regarded as deserving of an examination and overhauling

by a clinician. By both Schneider and Scott (6) the Crampton test was found unsatisfactory, while the newer method was justified by a comparison with clinical findings. In private conversation Schneider has expressed the opinion that his method was not likely to be a valid one as a test for a single day's fatigue, but would be indicative of cumulative fatigue.

The results that we have obtained in using these two tests are presented in table 3.

The figures indicate that for both the tests our subjects were in moderately good physical condition. The morning readings averaged between 43 and 49 with the Crampton percentage and between 11 and 12 with the Schneider rating.

There is on the rest days a merely nominal quantitative increase, but on the work days this has given place to a more marked decrease, amounting to 11 points, or 26 per cent, with the Crampton, and 3 points, or 27 per cent, with the Schneider index. At first sight this would seem to be a genuine fatigue effect. But let us examine the daily records. Here we see that, with the Crampton index, of the twenty-nine work days only 22, or 76 per cent, revealed a decrease, while the remainder showed either a rise or no change. Moreover, of the rest days, 36 per cent also showed a decrease. The balance in favor of a fall caused by the work performed is not great and would not indicate the index as a reliable criterion of daily fatigue. The figures are somewhat more favorable for the Schneider test. Here twenty-four work days, or 83 per cent, revealed a decrease, but even this does not appear strikingly significant, when it is seen that on 28 per cent of the rest days there was also a fall. The results would seem to exclude both tests as criteria of the physical fatigue resulting from the day's work of a single individual or a small group.

The two indices on the mornings after the work days showed no persistence of the fatigue of the day before.

With both the tests under consideration, but especially that of Crampton, we have been puzzled by the sudden appearance of indices widely divergent from the average. Thus, subject D, with an average Crampton index of 40 for the afternoons of the rest days, gave on one occasion a rating of 70 and on another one of 10—the former being due chiefly to an unusual rise of systolic pressure on standing, the latter to an abnormal lowering of this postural pressure. There seems to be no way of accounting for these divergences, which occurred in each case at the end of a quiet and physically restful day of study in the

TABLE 3

The Crampton and Schneider indices

Arithmetic means of all observations expressed in terms of nearest whole number

PERIOD	NUMBER OF OBSERVATIONS	CRAMPTON INDEX			SCHNEIDER INDEX		
		Amount	Difference	Number and percentage of days and character of effect	Amount	Difference	Number and percentage of days and character of effect
Rest days a.m.	64	49			12		
Rest days p.m.	64	51	+ 2	35 (55%) Increased	13	+1	35 (55%) Increased
				23 (36%) Diminished			18 (28%) Diminished
				6 (21%) Unchanged			11 (17%) Unchanged
Work days a.m.	29	43			11		
Work days p.m.	29	32	−11	22 (76%) Diminished	8	−3	24 (83%) Diminished
				6 (21%) Increased			3 (10%) Unchanged
				1 (3%) Unchanged			2 (7%) Increased

laboratory. The same subject, on a certain morning after a day of rest, showed an equally unexpected Crampton index of 6, likewise caused apparently by an abnormal fall of pressure on standing. Subject C, with an average Schneider index of 9 for the mornings of the rest days, gave an index of 5 on two occasions, due to a low rating on most of the component elements of the test. In none of these aberrant low indices did fatigue appear to be a factor, but their sudden appearance in apparently non-fatigued states makes one cautious about ascribing low indices in fatigued states to the concomitant fatigue. Of two individuals, at a given moment, one may be just above, the other just below, the critical border-line that exists for each component item of the test, and the one must receive a good, the other a poor, rating for that item. Whether the lowering influences are merely adventitious or temporary, or on the other hand have an important and persistent physical basis, might be revealed by several repetitions of the test at not too frequent intervals. But a test for fatigue that must be repeated several times at not too frequent intervals is not a practicable test for general use.

That, on the other hand, both indices are probably useful in the detection of physical depression accompanying pathological conditions is supported by one instance that came within our observation. Subject B showed an average Crampton index for the mornings and afternoons of the rest days of 48 and 50, and an average Schneider index of 13 and 12, respectively. On one day he exhibited symptoms of a mild infection, but spent the day quietly in the laboratory studying. His bodily temperature varied during the day between 37.6°C. (99.6°F.) and 38.8°C. (101.8°F.). His respective morning and afternoon Crampton ratings were only 30 and 15, and his Schneider ratings 3 and 4. The case was diagnosed by the subject's physician as a mild influenzal attack, and this rendered him unsuitable for our purposes for a period of two more days. It is unnecessary to say that the results of these days' tests were omitted from our summaries.

We have thought that the case for the Schneider test might perhaps be improved if a different rating were given to some of the data, especially the time of recovery following the stated exercise, and we have therefore attempted to devise a more highly differential system of evaluating these figures. But this has failed to give a substantially different result.

Further light is thrown upon the applicability of these two tests to the problem of daily fatigue by an examination of the question whether

there is any correlation between the magnitude of the two indices and the time after work at which the measurements are made. On several days we repeated the cardiovascular measurements on two subjects, B and D, at intervals within two hours after they had completed their daily walk. In most such cases the tests were made three times, and we have grouped the results into three successive periods following the

TABLE 4

Crampton and Schneider indices in successive periods following work

C = Crampton index. S = Schneider index

DATE MAY	SUBJECT	A.M.; BEFORE WORK		P.M.; AFTER WORK					
				10–29 minutes		30–59 minutes		60 and more minutes	
		C	S	C	S	C	S	C	S
2	B	40	12			35	8	50	11
4		45	15	20	6	18	7	49	11
10		59	9	34	4	19	7	20	9
11		42	10	20	4	25	6	37	7
12		65	9			50	8	45	8
17		36	7	−5	6	30	5	26	5
18		45	11	25	6	40	4	25	10
Averages.....		47	10	17	5	31	6	36	9
2	D	30	9					30	5
4		55	11			20	8	20	10
10		6	9			10	4	8	4
11		45	8			35	7	19	7
12		40	10	5	8	10	8	15	3
15		53	13	50	8	34	6		
16		36	13	65	9	35	8		
17		28	12	15	6	−8	8	20	8
Averages.....		37	11	34	8	19	7	18	6

end of the walk by ten to twenty-nine minutes, thirty to fifty-nine minutes, and sixty and more minutes, and have determined the indices for each of these periods. Since the component elements of the indices proved to be very variable we shall not burden the reader by detailing them, but shall confine ourselves to the indices themselves. The individual results are presented in table 4.

The averages of the figures show that each subject was consistent for himself, but that the two showed diametrically opposed tendencies.

Thus, during the successive periods after work subject B shows a progressive improvement of both indices, subject D a progressive deterioration. But an examination of the individual cases reveals great variability. For example, subject B shows on May 4 a fall in the Crampton index, from the morning reading of 45, to 20 in the first period after work, and to 18 in the second period, and a return to 49, 4 points above the morning index, in the third period. If the index reveals the subject's fatigue, does this mean entire recovery and more, within the second hour after the completion of the work? The same question applies to the Schneider rating of the same subject on May 10. Subject B shows on May 12 in the first period after work a Schneider index of 8, a fall of 2 points from his morning index, but in the third period an index of only 3. Does this mean that he is far more fatigued after an hour's rest than immediately after the completion of his strenuous task, a walk of 15 miles? Sometimes there is a progressive improvement in a subject's condition, as revealed by one or the other index in the successive periods after work; sometimes there is improvement followed by deterioration; sometimes there is only progressive deterioration. The table presents perhaps the most conclusive evidence that we have yet found that neither test is valid as a test for daily physical fatigue.

We should emphasize that we are not concerned with these two tests as means of detecting pronounced physical deterioration resulting from whatever cause, disease, overwork, use of drugs, even cumulative fatigue. In this field the Schneider test certainly, and the Crampton index probably, have demonstrated their value. What we are concerned with is the applicability of the tests in the detection and measurement of the fatigue of the day resulting from strenuous physical exertion. Here we believe they fail.

5. The product of the pulse pressure by the pulse rate. Following the suggestion by Erlanger and Hooker (7) in 1904, the product of the pulse pressure by the pulse rate is often interpreted as indicating the relative velocity of the blood flow. The summarized results of our observations here are presented in table 5.

It is here seen that a preponderance of both rest and work days exhibited a diminution in the rate of the blood flow in the afternoon, and there is no significant difference as between rest and work. The numerical decrease is not great, and in one of the four conditions, that of the afternoon work day, reclining position, there were sufficiently large figures on certain of the "rise" days to give the average a plus sign.

TABLE 5

The product of the pulse pressure by the pulse rate

Arithmetic means of all observations expressed in terms of nearest whole number

PERIOD	NUMBER OF OBSERVATIONS	PULSE PRESSURE BY PULSE RATE					
		Reclining			Standing		
		Amount	Difference	Number and percentage of days and character of effect	Amount	Difference	Number and percentage of days and character of effect
Rest days a.m.	64	2600			2607		
Rest days p.m.	64	2501	−159	43 (67%) Diminished	2499	−108	36 (56%) Diminished
				21 (33%) Increased			26 (41%) Increased
				0 Unchanged			2 (3%) Unchanged
Work days a.m.	29	2881			2961		
Work days p.m.	29	2906	+ 25	15 (52%) Diminished	2837	−124	19 (66%) Diminished
				13 (44%) Increased			10 (34%) Increased
				1 (4%) Unchanged			0 Unchanged

TABLE 6

Cardiac energy and peripheral resistance

PERIOD	NUMBER OF OBSERVATIONS	CARDIAC ENERGY				PERIPHERAL RESISTANCE			
		Reclining		Standing		Reclining		Standing	
		Average	Number and percentage of days and character of effect	Average	Number and percentage of days and character of effect	Average	Number and percentage of days and character of effect	Average	Number and percentage of days and character of effect
Rest days	64	Unchanged	46 (72%) Unchanged	Unchanged	43 (67%) Unchanged	Increased	34 (53%) Increased	Increased	35 (55%) Increased
			13 (20%) Diminished		11 (17%) Increased		17 (27%) Diminished		18 (28%) Diminished
			5 (8%) Increased		10 (16%) Diminished		13 (20%) Unchanged		11 (17%) Unchanged
Work days	29	Unchanged	20 (69%) Unchanged	Unchanged	21 (73%) Unchanged	Diminished	13 (45%) Increased	Unchanged	14 (48%) Increased
			6 (21%) Diminished		5 (17%) Diminished		13 (45%) Diminished		8 (28%) Unchanged
			3 (10%) Increased		3 (10%) Increased		3 (10%) Unchanged		7 (24%) Diminished

The percentages of the days on which a fall occurred were never great, ranging in the four conditions between 52 and 67. It is obvious that the velocity of the blood flow affords no criterion of daily fatigue.

Erlanger and Hooker, following the lead of Beaunis, have published a convenient table by which, from the constancy, the increase, or the diminution of the two factors, the diastolic pressure and the product of the pulse pressure by the pulse rate, inferences may be drawn with reasonable accuracy as to the two causative factors, the kinetic energy of the heart and the peripheral resistance. By the aid of this table we have evaluated our data with the results presented in table 6.

This table reveals the fact that in approximately 70 per cent of all the cases the energy of the heart is unchanged as between morning and afternoon. Moreover—and here is the striking and important feature—even on the work days the cardiac energy is similarly constant.

Occasionally, whether the individual has been resting or working, there appears to be a diminution, just as occasionally also there appears an increase, in heart energy. We have examined all these cases designated as diminution and find that a slight quantitative difference in some one of the original data, such as, for instance, a mere difference of one millimeter in diastolic pressure in a third of the cases,—a difference easily within the limits of error—would have so changed the rating as to indicate an unchanged instead of a diminished cardiac energy. Thus this evidence for an occasional diurnal diminution of this factor in physical efficiency falls to practically nil, and it may be confidently stated that the performance by healthy individuals of a day's physical work, such as a walk amounting to 14 miles and sufficiently intensive to cause pronounced sensations of fatigue, does not diminish the energy of the body's most indispensable organ.

The relation of rest and work to the peripheral resistance to the circulation of the blood is not so uniform. While the averages of our figures show that on the rest days this resistance is increased in the afternoon as compared with the morning, examination of the daily records reveals that such increase occurs in only slightly more than one half the days for both bodily positions; on the other days the resistance is diminished or unchanged. On the work days there is even greater discrepancy between the average and the day's records: In the reclining position the average shows a diminution, but such diminution is found in only 45 per cent of the days, while in another 45 per cent there is an increase; in the standing position the average shows no change, but such a condition is actually found in but 28 per cent of the

days. With such discrepancies no causal relation between fatigue and peripheral resistance is obvious.

6. The electrocardiogram. On one of our work days we made electrocardiograms, through the kindness of Professor Williams, of two of our subjects, B and D, before and after their walks of 16.75 and 15 miles respectively. With subject D there were no noticeable differences between the two records. With subject B too there were no observable differences except in the T wave. In the morning records it was wholly positive in leads I and II, while in lead III the positive phase was lower and was sometimes preceded by a slight negative phase. After the exercise the T wave consisted mainly of a negative phase, followed in most beats by a small positive one.

The diminution of a positive T wave or its actual reversal into a negative one under different conditions has been pointed out by various investigators, and, in accordance with a somewhat prevalent tendency to regard it as an index of cardiac power, its diminution or reversal might perhaps be interpreted as a weakening of cardiac energy. From the two cases which we here report and the lack of agreement among those best capable of judging as to the physiological significance of the T wave, it is idle to draw conclusions here. It may, however, be added that our records from the Beaunis-Erlanger-Hooker table show that with subject D, with an unchanged T wave, the cardiac energy on the day on which the electrocardiographic records were made, was unchanged, while with subject B, with a reversed T wave, the cardiac energy in the reclining position was unchanged and in the standing position was even increased. It is unwise to say more here, but the subject perhaps deserves further investigation.

7. The vascular skin reaction test. The vascular skin reaction, first described by Marey, is induced by stroking the skin of the forearm by a blunt instrument made for the purpose, which exerts a uniform pressure. There results a white streak, appearing within a few seconds, gradually intensifying, and then gradually fading away. The time relations of the picture vary, and Ryan (8), observing that the time that elapsed between the moment of making the stroke and the beginning of the fading of the streak appeared to be decreased by work and activity and increased by rest or sleep, proposed to use this "fading time" as a criterion of fatigue. No considerable study of the test on normal individuals has ever been published. King (9) used it on fifty-four soldiers suffering from irritable heart, and found both a diurnal decrease on rest days and a decrease induced by light "setting-up"

exercises; influenzal patients showed a forenoon decrease and an afternoon increase. He concludes that the test is of value in the quantitative estimation of fatigue in clinical conditions, but that "owing to the various sources of possible error in the test it is more suitable for group study than for individual cases."

Coincident with our other observations we have applied the vascular skin reaction test to our four subjects, both forenoons and afternoons, immediately following the completion of the respiratory tests. Two observers each with a stop-watch, simultaneously noted the "latent period," or the time that elapsed between the moment of making the stroke and the moment of definite appearance of the white streak, and the "fading time." The succession of events following the application of the stroke was usually gradual, one passing almost imperceptibly into another, and hence it was often difficult to determine the moments exactly. Occasionally the white streak seemed to appear simultaneously with the stroke and to persist, beginning after some seconds, however, to increase in intensity. Occasionally momentary and misleading fluctuations in the intensity of the streak were observed, obscuring the moment of maximum whiteness. Whenever, as occasionally happened, the test was repeated several times in succession within a few minutes, the observed times often varied enormously. Notwithstanding these difficulties each measurement as noted on our records represents substantial agreement between the two observers and, it is believed, as high a degree of accuracy as two careful, experienced and conscientious observers are capable of in the presence of an almost imperceptibly graded succession of events. The results are presented in table 7.

The results are remarkable for their uniformity. The average shortening of only one second in the fading time on the work days hardly shows the method to be a valid test for the daily fatigue resulting from such physical exertion as was performed by our subjects. The coefficients of variation for both the latent period and the fading time were computed for subject A and were found to be low, namely, 0.21 for the former and 0.19 for the latter. Between the two there was also a very low coefficient of correlation, namely, 0.16, indicating that, if one is significant, the other is not.

9. Conclusions from cardiovascular data. We have thus been unable to find a reliable criterion of daily physical fatigue in any one of the phenomena we have studied: The rate of the heart beat in the reclining or the standing bodily position, the increase in rate on passing from one

to the other, the immediate increase in rate after a given exercise, the time required after the given exercise for a return to the original rate, the reclining or the standing systolic or diastolic blood pressure, the decrease in the systolic pressure on passing from the reclining to the standing position, the pulse pressure in the two bodily positions, the combination of these various data as employed by either the Crampton or the Schneider test, the relative velocity of the circulating blood in either the reclining or the standing position, the kinetic energy of the heart, the peripheral resistance to the circulation of the blood, and the vascular skin reaction.

Of the two subjects who gave us the largest number of data subject B was weaker muscularly, less trained physically, but more stable nervously, than subject D. Neither one, however, revealed a greater

TABLE 7

The vascular skin reaction

Arithmetic means of all observations expressed in terms of nearest whole number

PERIOD	NUMBER OF OBSERVATIONS	LATENT PERIOD IN SECONDS		FADING TIME IN SECONDS	
		Amount	Difference	Amount	Difference
Rest days a.m.	65	11		33	
Rest days p.m.	65	11	0	33	0
Work days a.m.	29	11		32	
Work days p.m.	29	11	0	31	−1

tendency on work days toward a fatigue response to the various cardiovascular tests, except in the Crampton and Schneider ratings, where B's actual fall was twice that of D, although his percentage fall was not so marked.

The question then arises: Is it to be expected that in the future a criterion of daily physical fatigue will be found in any of the physical phenomena of the cardiovascular system? Here we are obliged to be sceptics. The cardiovascular system, serving as it does the needs of the whole body, ought, in the interests of the organism, to maintain its efficiency and its capacity for work unimpaired so long as it is possible. Observations, ours and others', show that this indispensable condition is maintained, even after strenuous demands are made upon this physiological system. It is sensitive and constantly reacts to a host of intrinsic and extrinsic influences. Where these reactions are per-

sistent and leave a continuing impress they may be detected by appropriate tests, and here some of the tests of which we have made use in our investigation appear to have proved practicable and valuable. But in the ordinary affairs of life the reactions are temporary and fleeting. We are then forced to ask whether it is not futile to search longer among the physical manifestations of cardiovascular phenomena for an objective test for daily fatigue.

The marked effect of psychic conditions upon the cardiovascular system, especially the heart rate, is a factor that cannot be controlled or kept constant, and this, in our subject C especially, made it difficult to secure uniformity in results. Even after a week of tests twice daily he often was so visibly nervous that we were convinced that it affected his ratings in the cardiovascular tests markedly. This in itself makes it practically impossible to use these tests for such an individual's fatigue from a day's work, although they might be valid in the average of a large group of workers.

II. Respiratory efficiency tests. In 1918 Lieut. Col. Martin Flack (10), Secretary of the Air Medical Investigation Committee of Great Britain, reported the results of the employment of several respiratory tests which had proved valuable in detecting the incipient stages in the breakdown of aviators suffering under the stress of flying. Although the "fatigue" of the aviator of which the Report frequently speaks is more serious than, and probably of a different nature physiologically from, the fatigue of a man who has just completed a 14-mile walk, however unaccustomed he may be to such physical exertion, we thought it well to include the tests in our own experiments. They were made immediately after the completion of the cardiovascular tests and, on the work days, they began approximately one and a quarter hours after the men had returned from their walk. The tests comprised the following, which the subject took in the order named:

1. The duration of the period of holding the breath after a full expiration and inspiration.
2. The vital capacity.
3. The amount of the supplemental air.
4. The expiratory force, measured by the maximum height to which the individual is able, after a full expiration and inspiration, to blow the column of mercury in a manometer.
5. The duration of the period of maintaining by his respiratory muscles, after a full expiration and inspiration, the column of mercury in a manometer at a height of 40 mm.

6. The rate of the pulse at the beginning, middle and end of test no. 5.

It is unnecessary to specify here further details in the performance of the tests. In measuring the expiratory force and the sustaining of the mercury column certain technical difficulties were overcome by devising glass mouthpieces of the proper size and shape. The four subjects underwent the tests with interest and zest and evidently endeavored to give their best results. The arithmetic averages of the data are presented in table 8.

Here the only difference between rest and work days that is of sufficient magnitude to arrest the attention is the surprising increase in the

TABLE 8

Respiratory efficiency tests

Arithmetic means of all observations expressed in terms of nearest whole number

PERIOD	NUMBER OF OBSERVATIONS	BREATH HELD		VITAL CAPACITY		SUPPLEMENTAL AIR		EXPIRATORY FORCE		SUSTAINING Hg.	
		Seconds	Difference	Cc.	Difference	Cc.	Difference	Mm Hg	Difference	Seconds	Difference
Rest days a.m.	65	75		4071		1691		174		62	
Rest days p.m.	65	79	+ 4	4098	+27	1698	+ 7	176	+2	63	+1
Work days a.m.	29	75		4167		1745		192		59	
Work days p.m.	29	87	+12	4164	− 3	1721	−24	186	−6	61	+2

length of the breath-holding period. This appears to have been caused by subject D, whose performances in this respect were sometimes extraordinary. On four of his fourteen work days his afternoon figures were 128, 130, 149 and 158 seconds respectively. If these anomalous figures be omitted from the calculations, the afternoon average would be 78. With this correction there appears to be nothing in the respiratory data to justify the selection of these physiological manifestations as criteria of daily physical fatigue. The same conclusions may be drawn from the individual records of the two chief subjects, B and D.

It might be mentioned incidentally that subject D was able to make extraordinarily high readings for expiratory force: His average for the mornings of the work days was 242 and for the afternoons 233; he sometimes reached over 250 and once 278. The other subjects were more

uniform and made much lower records, seldom going much over 160 mm. Hg.

Flack found that during the period in which the column of mercury was being maintained at 40 mm. the heart rate of the normal individual, counted in successive 5-second periods, rose either gradually to a maximum or quickly to a maximum, where it was sustained. In individuals suffering from flying stress, on the other hand, the rate jumped quickly to a maximum and then fell away to normal or even below normal. We found, not the high rates quoted by Flack, but merely nominal changes in rate with their signs varying greatly. After neither rest nor work was there a fall, except in a few instances.

III. THE RESISTANCE STRENGTH TEST. The "resistance strength test," or "spring balance muscle test," was devised by Martin (11) in 1915 for the purpose of measuring the muscular impairment of persons, chiefly children, afflicted with infantile paralysis. It proved its value in this field and later was used in contributing valuable knowledge of the strength of various types of men and women, of the strength problems of industrial work, and of muscular impairment in soldiers. Martin has never maintained that the test can profitably be used to demonstrate or measure the daily fatigue of the individual, and says frankly that his industrial studies "suggest that although the strength showing is not to be looked upon as indicating invariably the presence or absence of fatigue in individuals on single days, it is a fairly reliable index of fatigue of groups or of individuals over a long period."

Since a test which has proved so successful as has Martin's in certain fields will almost surely be employed sooner or later in the attempted detection of the diurnal physical fatigue of individuals, and since few, if any, data bearing on this had been published, we thought it desirable to use the test with three of our subjects. In the order of application it followed immediately after the respiratory tests in both forenoon and afternoon, and on the work afternoons began approximately one hour and twenty-five minutes after the subjects had completed their walk. The test consists in overcoming, by means of the pull of a graduated spring balance in the hands of an operator, the contractions of five selected groups of muscles on each side of the body of the subject and noting the strength of pull in pounds or kilograms required for each. The sum of these ten figures, multiplied by the constant 5.65 (for men) gives the total strength of the individual. The arithmetical averages of the detailed measurements, expressed in terms of the nearest whole number, are presented in table 9. The total strength is calculated, not from these

averages, but, more correctly, from the totals of the observed data. In making all of these observations the same person acted as operator throughout.

It is obvious that the resistance strength test cannot be used as a measure of daily physical fatigue, even though caused by a 14-mile walk, for a small number of individuals. The increase of 52 pounds for rest days and of 17 pounds for work days is too slight to be significant of any physiological change, and an increased muscular strength after the expenditure of much muscular energy is hardly to be looked for. The

TABLE 9

Muscular strength in pounds

Arithmetic means of all observations expressed in terms of nearest whole number

R.P. and L.P. = Right and left pectorals
R.W. and L.W. = Right and left wrist flexors
R.F. and L.F. = Right and left forearm flexors
R.Ad. and L.Ad. = Right and left thigh adductors
R.Ab. and L.Ab. = Right and left thigh abductors

PERIOD	NUMBER OF OBSERVATIONS	R.P.	L.P.	R.W.	L.W.	R.F.	L.F.	R. Ad.	L. Ad.	R.Ab.	L.Ab.	TOTAL STRENGTH	DIFFERENCE
Rest days a.m.	45	95	89	46	43	80	76	50	51	55	56	3628	
Rest days p.m.	43	97	91	45	42	79	76	50	54	58	58	3680	+52
Work days a.m.	29	100	94	46	42	81	77	52	53	57	58	3773	
Work days p.m.	29	103	96	46	44	83	78	52	54	57	58	3790	+17

mornings after work exhibited also no material differences from other mornings. The summaries of the records of individual subjects are also not significant, for subject B showed, for rest and work days respectively, +29 and −16; subject C, −2 and +107; and subject D, +99 and +124.

The coefficients of variation of the total strength of the two subjects were computed for the mornings and afternoons of the work days and were found to be very low, namely, for subject B 0.05 and 0.07 and for subject D 0.04 and 0.04 respectively.

It is stated above that all the data which were used in the compilation of table 9 were obtained by one operator. This strict limitation to one operator was due to the fact that on one occasion when the usual operator was absent another man acted in his capacity. It was observed that the measurements made by him were considerably below

the usual measurements. On the following day both operators made the test independently on two subjects with the result that the figures obtained by the temporary operator were less by 8.6 per cent with subject B and by 12.5 per cent with subject D than those obtained by the usual operator. Both operators were familiar with the test and believed that they were following the required procedure exactly. It was found, however, that with several of the muscle groups the directions of the pulls by the two operators were different, and this may have been responsible for the differences in the measurements. In a study of the resistance strength test Muscio observed also a consistent difference in the figures obtained by two operators and came to the general conclusion that the results of the test were partly a function of the operator.

IV. SUMMARY

We have examined experimentally a considerable number of physical tests which either have been proposed, or might conceivably be employed, as indicators of fatigue in human beings. We do not find a reliable criterion of the daily physical fatigue of the individual, resulting from such physical exertion as a walk of fourteen miles, in any of the tests, which comprise:

1. The rate of the heart beat in the reclining or the standing bodily position; the change in rate on passing from the reclining to the standing bodily position; the immediate increase in rate after a given exercise; and the time required after a given exercise for a return to the original standing rate.

2. The systolic or the diastolic blood pressure in the reclining or the standing bodily position; the change in the systolic pressure on passing from the reclining to the standing position; and the pulse pressure in either of the two positions.

3. The combination of several of the above cardiovascular data in the test of Crampton.

4. The combination of several of the data in the test of Schneider.

5. The velocity of the blood flow as indicated by the product of the pulse pressure by the pulse rate in either the reclining or the standing bodily position.

6. The kinetic energy of the heart or the peripheral resistance to the circulation of the blood, as indicated by the Beaunis-Erlanger-Hooker table.

7. The electrocardiogram.

8. The vascular skin reaction, as employed by Ryan.

9. The duration of the period of holding the breath, the vital capacity, the amount of the supplemental air, the expiratory force, the duration of the period of maintaining by the respiratory muscles a column of mercury at a height of 40 mm., and the rate of the heart beat during such maintenance—as employed by Flack.

10. The resistance strength test of Martin.

Several of the above tests have demonstrated their value in the detection of pronounced physical deterioration amounting often to a pathological condition, or of fatigue in masses, but none of them appears to be practicable in the detection or measurement of the physical fatigue resulting from the day's work of the individual.

BIBLIOGRAPHY

(1) Muscio: Brit. Journ. Psychol. (Gen. Section), 1921, xii, 31; Repts. of the Industrial Fatigue Research Board, 1922, no. 16, 38.
(2) Weysse and Lutz: This Journal, 1915, xxxvii, 330.
(3) Crampton: Med. News, 1905, lxxxvii, 529; N. Y. Med. Journ., 1913, xcviii, 916.
(4) New York State Commission on Ventilation: Amer. Journ. Pub. Health, 1915, v, 85; final report in press. See also Lee and Edwards: Proc. Soc. Exper. Biol. and Med., 1915, xii, 72.
(5) Schneider: Journ. Amer. Med. Assoc., 1920, lxxiv, 1507.
(6) Scott: Journ. Amer. Med. Assoc., 1921, lxxvi, 705.
(7) Erlanger and Hooker: Johns Hopkins Hosp. Repts., 1904, xii, 145.
(8) Ryan: Proc. Amer. Physiol. Soc., This Journal, 1918, xlv, 537.
(9) King: Arch. Int. Med., 1919, xxiii, 527.
(10) Flack: Repts. of the Air Medical Investigation Com., 1918, no. 3.
(11) Martin: Pub. Health Repts., 1920, 1895; Physiol. Reviews, 1921, i, 454.

STUDIES ON THE MECHANISM OF STERILIZATION OF THE FEMALE BY SPERMOTOXIN

J. L. McCARTNEY

From the Hull Physiological Laboratory, University of Chicago

Received for publication October 12, 1922

A number of workers report the production in the animal body (male and female), of specific and non-specific spermotoxin on the subcutaneous or intravenous injection of spermatozoa. Others have reported temporary sterility both of the male and the female following similar injections of sperm. There is also some evidence that in normal coitus some of the spermatozoa may penetrate the tissues of the genital tract of the female, and may in consequence act as antigen. The present work was undertaken at the suggestion of Doctor Carlson with the view of elucidating the mechanism or nature of the female sterility induced by immunization with spermatozoa.

LITERATURE. Spermotoxins were produced in 1899 simultaneously by Metchnikoff (1) and Landsteiner (2). The former produced an isospermotoxic serum. Taylor (3) found that no spermotoxic serum could be obtained by immunization with isolated nucleic acid, protamines, or ether extracts of sperm, although he also found that immunizing with whole sperm, an active anti-serum was obtained. Landsteiner (2) and Dunbar (4) report that their spermotoxins immobilized and killed (without causing solution) the spermatozoa in a hanging-drop preparation. De Leslie, as quoted by Ricketts (5), rendered male mice sterile for 16 to 20 days by the injection of spermotoxic serum, and thus concluded that the amboceptors are taken up by the homologous cells in the living animal.

Metchnikoff (1), and Ricketts (6) state that spermotoxic serum is hemolytic, and from this they conclude that certain receptors are common to erythrocytes and spermatozoa, but hemolytic serum is not spermotoxic. Kolmer and Pearce (7) state that the hemolytic property of the cytotoxic serum is likely due to the presence of blood in the antigen. Ricketts (6) says (p. 296) that castrated animals will yield spermotoxin by immunization with spermatozoa, showing that the

amboceptor is not of necessity produced by analogous tissue in the immunized animal, and he further states that immunization with the plasma of ova causes the formation of a spermotoxic amboceptor.

Kohlbrugge (8) reports that in the normal copulation of rats and other rodents, the spermatozoa penetrate the epithelium of the generative mucosa and invade the underlying connective tissue. If this takes place in women, the commonly found sterility of professional prostitutes may be due in part to spermotoxins (9). Walstein and Ekler (10) report that in rabbits a distinct Abderhalden reaction for testicular proteins may be obtained shortly after coitus. Metalnikov and Strenikov (11) have reported that when spermatozoa are placed in the body tissues, a spermotoxin is produced, although the antigen may be implanted in the tissues as an entire testes graft, or enclosed in a collodion sac. Herrmann and Stein (12) state that immune serum can be obtained against the spermatozoa, but that the immunity is not specific. Kolmer (13) states that antispermotoxin may be produced by immunization with a spermotoxic serum.

Westermark (14) has advanced the theory that many cases of sterility in women are due to a degeneration of the ova (due to luxurious mode of life, genius or inheritance), setting up a negative chemotaxis, which repels the spermatozoa.

Dittler (15) found that by immunizing female rabbits with progressive doses of rabbit ejaculation fluid, they were made sterile for a period of 4 months or longer. He immunized the rabbits by injecting into the ear vein the fluid which he withdrew from the vagina after copulation, and which he gave in small doses at intervals of 1 to 8 days, repeating the injections 2 to 10 times, until a total of 2 to 5 cc. had been given. He continued the injections until the antibody was found in the blood. This antibody was usually present in the blood in 6 to 8 days after the injection of the antigen. He also found that the immunization did not hinder ovulation, nor in any way affect the habits or behavior of the rabbits. There was an initial loss of body weight after the injections, but this was regained. No anaphylaxis was noted in his rabbits. Dervieux (16) claims an individual specificity for spermotoxins. Dittler (15) states that although he produced a specific toxin to human spermatozoa and a specific isospermotoxin in the rabbit, and showed this by agglutination tests, he could show no individual specificity.

Moore (17) has observed that the presence of testicular grafts has no demonstrable deleterious influence upon the somatic or psychical characteristics of the female animal. He further says that no indicated

antagonism can be shown between the ovary and testis, even when functioning in the same animal. The transplanted testis does not interfere with pregnancy in the female so grafted; and Moore has had several such rats that have borne normal young. He has shown microscopically that although the testis graft apparently functions normally as to internal secretions, the germinal cells in the graft degenerate, and no spermatozoa are formed after transplantation.

In a recent article Guyer (18) makes the following conclusions from his experimental findings:

1. "Spermotoxic sera, prepared by injecting fowls repeatedly with the spermatozoa of rabbits, are toxic in vitro for the spermatozoa of both rabbits and guinea pigs."

2. "When introduced into the blood stream of male rabbits at intervals for 4 or 5 weeks, such serum produced partial or complete sterility. Inactivation of many spermatozoa, reduction in number, or even complete disappearance from the semen occurred."

3. Microscopical examination of the testis of a serum-treated male showed disintegrative changes taking place in the seminiferous tubules.

4. "The blood serum of a rabbit injected intravenously with its own spermatozoa becomes highly toxic for the spermatozoa of rabbits, including its own."

5. "The spermatozoa of a rabbit which has been repeatedly injected with its own semen are much less viable, both in normal rabbit serum and in spermotoxic serum, than are normal spermatozoa."

Hektoen (19), on the basis of his recent work, and Dittler (15), both conclude that spermotoxins are specific. Hektoen found that a specific precipitin serum for human semen can be produced, and may be even "semen-specific." He further showed that extracts of carefully washed spermatozoa give precipitates with antisemen serum.

EXPERIMENTAL METHODS. The albino rat was chosen for our experimental work on breeding, because of its fecundity. In this animal ovulation occurs at intervals of about 3 weeks, and begins when the rat is about 2 months old. The female begins to breed when she is 90 to 100 days old, and in all our experiments we allowed the rats to cast one litter before immunization. We then took the young away from the mother within 24 hours, and began the immunization treatment. One female of each litter was used as a control.

In order to study the effect of past immunity on later fecundity, the immunized rats that finally gave birth to litters were taken away from their young and mated the next day, and the fecundity noted.

To determine the toxic effect of the antigen injection on existing pregnancy, a series of thirteen female rats in advanced pregnancy was subcutaneously injected, and the effects studied.

To determine if the effects of immunization with sperm fluid are transmitted to the offspring, two litters from females that had shown periods of delayed pregnancy, and two litters from pregnant females that had been injected with sperm-fluid before the end of term, were raised to maturity. Four individuals from each litter were selected, consisting of two males and two females. These were mated when they were 4 months old.

Chickens were used for the direct study of the ovum after the immunization. The eggs (laid in trap nests), were incubated for 72 hours, and the fertility determined by the development of the blastoderm. Six hens were used, and they were allowed to continually run with the rooster. Every hen was regularly laying fertile eggs before the immunization.

From the experimental findings of other workers, as reported in the literature, it was concluded that the spermotoxins are present in the blood of the immunized animal. In order to determine if they are present in the vaginal or uterine secretion of these same animals, small cotton swabs were inserted into the vagina of the rats, and these swabs were then tested for the antibody as follows: 2 cc. of sperm suspension (40,000 spermatozoa in each 1 cc. of Ringer's solution), were placed in clean agglutination tubes, and kept at 40°C. The moistened vaginal swabs were then placed in these tubes, and kept at the same temperature. The time when the spermatozoa agglutinated or clumped was noted by stop-watch. Hanging-drops were made, and the time when immotility of the spermatozoa took place was noted. The control tests were made, using the material from vaginal swabs from normal virgins and multiparous non-immunized rats.

In ten of the rats the cotton swabs were weighed before and after insertion into the vagina, and the amount of moisture secured determined. These swabs were then added to different amounts of Ringer's solution at 40°C., and one drop of these solutions was added to 1 cc. of sperm-suspension, as above. These tubes were then incubated at 40°C. for half an hour, and at the end of this time the degree of agglutination was read, and from this the strength of the spermotoxin determined.

Adult male rats were used to study the effects of the injection of spermatozoa on the testes.

Preparation of the antigen. The sperm-fluid (rat and chicken) was

obtained by aseptic removal of the testes, and after sectioning the testes the spermatozoa were shaken into sterile Ringer's solution. Where specific fluids were used only one testis of the rat was removed and the other was left to function in breeding tests, or to be removed later for the preparation of specific sperm-suspension.

The human sperm was collected by the usual clinical methods for collecting semen.

The control injections in the case of the rats were Ringer's solution, extracts of the salivary glands of the same rat from which the testes were removed. In the case of the chickens, the control injections were an extract of chicken liver.

Injection of antigen. The fluids were injected subcutaneously into the abdominal wall of the rats, and likewise in the hens. The usual aseptic care was taken in all injections, and only six or seven cases of superficial sepsis were noted in the 118 rats injected.

Since the normal rat ejects about 40,000 spermatozoa in a single copulation (19), we diluted the sperm-fluid with Ringer's solution, so that there was about this number of spermatozoa, by count, in each 0.5 cc. of the rat sperm-fluid injected. Ovulation in the rat usually regularly follows 20 to 48 hours after parturition, and judging from this we would regulate the injections of the antigen so that the last injection of sperm-fluid would be 6 to 8 days before her next ovulation. The doses of antigen were varied, 0.5, 1.0 and 1.5 cc. 4 days apart, but never exceeding a total of 3 cc. The female rat in the process of immunization was kept away from the males, and at the end of 21 days she was mated. Thus each female rat was mated 21 days after casting a litter, at a time when she was supposed to ovulate.

The hens were injected with 5 cc. of the rooster sperm-fluid at one dose, only slightly diluted with Ringer's solution.

Five adult male rats were injected subcutaneously with from 1 to 5 cc. of rat sperm-fluid or testes extract, and one male was injected with 1 cc. of human semen.

Criteria of immunity. The criteria of immunity after injection of sperm-fluid or testes extract were as follows:

1. Delayed pregnancy in the rat.
2. Infertility of the eggs (chickens).
3. Immobilization and agglutination of the sperm by the vaginal secretion of immunized rats.
4. Impairment of testes and sperm in *vivo* in the male rat.

RESULTS. We found no case where the sex behavior of the female

rat was apparently altered by the subcutaneous injection of spermatozoa. The immunized rats copulated with the male as did the normal or control rats. In the 118 injected rats one rat died of what appeared to be anaphylaxis, after it had been injected (first injection) with 1.5 cc. of the sperm-fluid.

Table 1 shows the results of 79 breeding tests, and it can be seen that by the injection of the sperm-fluid (either rat or human) or testes extract,

TABLE 1

Breeding test on the albino rat after subcutaneous injection of spermatozoa into the female

TYPE OF TREATMENT GIVEN THE FEMALE RATS	TOTAL NUMBER OF ANIMALS	NO. OF ANIMALS BECOMING PREGNANT	TIME IN WEEKS BETWEEN MATING AND CASTING OF FIRST LITTER			AVERAGE NUMBER OF YOUNG TO EACH LITTER
			Minimum	Maximum	Average	
Injected with rat sperm-fl.						
Specific	14	12*	6	22	11.7	5.6
Non-spec	25	5**	6	9	7.7	5.0
Injected with testes-ext.						
Specific	15	14†	6	22	11.1	4.6
Non-spec	4	4	4	21	12.5	5.0
Injected with human semen	5	3‡	4	17	9.0	5.0
Control—Salivary gl. ext	4	4	3	5	4.0	8.0
Control—Normal rats not inj	12	12	3	6	3.9	6.7

* One rat died, non-pregnant, in the 16th week after mating, and one at the end of observation, 22nd week, failed to show fertility.

** Two rats died, non-pregnant, and one pregnant, in the 4th, 5th and 9th week respectively. Eighteen rats showed no signs of fertility at the end of observation, 8th week.

† One rat at end of observation, 22nd week, showed no signs of fertility.

‡ Two rats died, non-pregnant, in the 7th and 10th week respectively.

the female rat was rendered sterile for a period of from 2 to 22 weeks or longer.

Our results confirm the findings of King and Stotsenburg (21) that the normal litter of rats averages 6.8 individuals, and from the above table it will be noted that this number is decreased in the immunized animals, when they finally become fertile.

Six female rats, which had been previously immunized with spermo-

toxins, and had shown a period of delayed fecundity, were taken from their young within 24 hours after casting their litters, and mated. The results of the repeated gestation after the period of immunity were as follows: One of the females cast a litter of 8 young in the 4th week after mating; three cast litters in the 5th week, two with 5 young and one with 6; and the other two females cast litters in the 7th week, each containing 2 young. All the young appeared normal.

The injection of pregnant rats with sperm-fluid showed five cases out of the thirteen which appeared to be abortions. Two cases on the 3rd day after injection of 1 cc. of sperm-fluid, gave birth to litters; the first rat casting 6 still-borns, and the second rat casting 9 young, which were undersized and did not live more than a day. The three other observed cases were as follows: The first rat began a foul-smelling, vaginal discharge on the 7th day after injection of 0.5 cc. of the sperm-fluid, and the animal became progressively thinner and appeared sick. This condition continued for a week, and then she began to improve, and in two weeks seemed to be completely recovered, although all signs of pregnancy were absent. The second rat, which had been injected with 1 cc. of the sperm-fluid, died on the 10th day after treatment. The cervix was found to be widely dilated, and the uterus contained 6 fetuses, partially decayed, which appeared to be in the 18th day of gestation. The third rat, which had been injected with 1.5 cc. of the sperm-fluid, began a foul vaginal discharge on the 12th day, began to lose weight and died on the 21st day. The cervix was dilated as above, and the uterus contained 9 putrefied fetuses. The uterus was greatly congested, and there was a general peritonitis. All the other 9 pregnant rats came to term in spite of the injection, and gave birth to normal litters.

In the experiment on the offspring of immunized females, no effect could be noted on the fecundity of the females or potency of the males because of the sperm-fluid treatment given the mothers. All the females, which were individually mated with one of the selected males, cast a litter of 5 to 8 young within 6 weeks after mating.

The experiments on chickens yielded the following results: The first hen, which was injected with sperm-fluid, laid in succession 8 fertile eggs after she had received the injection, and then laid 20 sterile eggs within the course of the next 45 days. During this time, on the 16th and 20th days, she laid fertile eggs. After the 45th day she laid 6 fertile eggs, and then we re-injected her with 5 cc. of rooster sperm-fluid. In the course of the next 33 days she laid first, 3 fertile eggs, then 12 sterile eggs, and on the 14th day after the second injection, a fertile

egg. After the 33rd day, this hen began again regularly to lay fertile eggs.

The second hen injected with sperm-fluid laid 9 fertile eggs in the first 16 days after injection, and then during the following 67 days she laid 28 sterile eggs, and then began regularly to lay fertile eggs.

The third hen laid 3 fertile eggs in the first 9 days following injection with sperm-fluid, and then 8 sterile eggs in the next 12 days, and then laid fertile eggs regularly for the next 27 days, at which time she was re-injected with sperm-fluid. She died 2 hours after injection apparently from anaphylactic shock.

The fourth hen laid 1 fertile egg in the 8 days following injection with sperm-fluid, and during the next 41 days she laid 9 sterile eggs and a fertile egg on the 7th, 13th and 26th days. She then laid 4 fertile eggs during the next week, and we re-injected her with sperm-fluid. During the following 8 days she laid 3 fertile eggs, and during the next 35 days she laid 14 sterile eggs, and a fertile egg on the 20th day. At the end of observation, 35 days after the second injection of sperm; she was still laying sterile eggs.

The fifth hen, which was injected with liver extract, laid 31 fertile (and no sterile) eggs during the 88 days of observations. The sixth hen, which was not treated in any way, laid 51 fertile (and no sterile) eggs during the 88 days of observation.

Spermotoxic (action of the vaginal secretion. By making swabs from the vagina of the immunized rats and putting them to the agglutination test, as above described, we found that the spermotoxin was present in the vaginal secretion. The vaginal secretion of the pregnant non-injected rats also appeared to contain spermotoxic properties, although not in such great concentration as in the secretion from the rats that had been injected with the sperm-fluid subcutaneously. Tables 2 and 3 show the results of the vaginal-swab tests.

It is well known that the vaginal secretion of normal females may be injurious to the spermatozoa, but it can be seen by the above table that the spermatozoa are much more rapidly immobilized and agglutinated by the vaginal secretion of the immunized animal than by that from a pregnant or a normal female rat. It is also to be noted that the antibody reaction appears to be proportional to the amount of antigen given in the immunization.

The concentration of the spermotoxin in the vaginal secretion was roughly determined as outlined above, and although the results are only relative, we may conclude that the antibody is present in sufficient

TABLE 2

Rats. Action of vaginal secretion on sperm suspension

TYPE OF TREATMENT GIVEN THE RAT BEFORE TESTING	TOTAL NUMBER OF RATS TESTED	TIME REQUIRED FOR SPERM TO BECOME IMMOBILE									TIME REQUIRED FOR SPERM TO AGGLUTINATE OR CLUMP								
		Minimum			Maximum			Average			Minimum			Maximum			Average		
		Hr.	Min.	Sec.	Hr.	Min.	Sec.	Hr.	Min.	Sec.	Hr.	Min.	Sec.	Hr.	Min.	Sec.	Hr.	Min.	Sec.
Immunized with 0.5 cc. specific sperm-fluid	10			30		10	—		3	13			50		15	—		3	57
Immunized with 1.0 cc. specific sperm-fluid	8			10			50			34			30		2	—		1	1
Immunized with 1.5 cc. nonspec. sperm-fluid	4			10			50			31			40		2	—		1	18
Immunized with 3 cc. nonspec. sperm-fluid	4			10			40			25			40		1	30		1	2
Normal pregnant female	5		8	40		11	—		9	33		10	10		10	20		10	15
Normal multipara	4		50	—	3	—	—	1	25	—		35	—	11	—	—	5	27	30
Normal virgin	4		40	—	6	—	—	2	53	—	—	—	—	—	—	—	—	—	—
Untreated sperm-susp.	2	11	—	—	20	—	—	15	30	—	—	—	—	—	—	—	—	—	—

TABLE 3

*Agglutination of sperm-suspension on the addition of one drop of diluted vaginal secretion from immunized rats**

DOSE OF ANTIGEN	UNDILUTED	3 TO 5 MGM. VAGINAL SECRETION DILUTED WITH RINGER'S SOLUTION					
		1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	10 cc.
0.5 cc. sperm-fl.	++++	++	++	+	+	−	
0.5 cc. sperm-fl.	++++	++	++	+	−		
1.0 cc. sperm-fl.	++++	++	+	+	−		
1.0 cc. sperm-fl.	++++	+++	+++	+	+	−	
1.0 cc. sperm-fl.	++++	++++	+++	++	+	±	−
1.5 cc. sperm-fl.	++++	+++	+	+	±	−	
1.5 cc. sperm-fl.	++++	+++	++	++	+	+	−
3.0 cc. sperm-fl.	++++	++++	++++	+++	+++	++	±
3.0 cc. sperm-fl.	++++	++++	+++	+	+	±	−

* Note: This test was also applied to a pregnant rat, and the vaginal secretion in this case was found to cause agglutination or clumping in dilutions up to 1:200.

strength to react in from 1:100 to 1:1000 dilutions, as is shown in table 3, which gives the dilutions as noted in 9 immunized rats.

In the series of six male rats studied, one showed no effect from the subcutaneous injection of testes extract. One rat, injected with 1 cc. of human semen, became sick on the following day. Within a week he became very passive, although otherwise apparently normal. He paid no attention to the females. His testes became retracted into the abdomen. This rat died 10 weeks after the injection. The testes were found atrophied and contained no spermatozoa. One male, injected with 2 cc. of rat sperm-fluid, showed enlarged testes for the first week, and then progressive decrease in size. He died 6 weeks after injection. The testes showed fatty degeneration, with very few, immotile, spermatozoa. One rat was injected with 5 cc. of rat sperm-fluid. At the time of injection the testes were 2.5 cm. in length by external measurement; two days later, they had increased to a length of 4 cm. and 19 days later 5.5 cm. There was a semen-like discharge from the penis, but this material contained no spermatozoa. The rat's hair was rough. The testes progressively decreased in size, and when examined 40 days after injection, the testes were decreased in size. About half the normal number of spermatozoa were present, but were all immotile. The two other rats, each injected with 2 cc. of rat sperm-fluid, showed enlargement of the testes for the first 2 weeks, but within a month the testes had returned to normal size. On examination, 7 weeks after injection, the testes appeared normal although they did not contain as large a number of spermatozoa as normally. These were motile.[1]

SUMMARY AND CONCLUSIONS

1. Female rats may be sterilized for a period of 2 to 22 weeks by subcutaneous injections of spermatozoa, or testes extract (confirming the work of Guyer, and Dittler on rabbits). Subcutaneous injections of other organ extracts do not cause sterility.

2. The sterility seems to be due to the presence of spermotoxins in the vaginal and uterine secretions of the immunized animal. These spermotoxins immobilize and agglutinate the spermatozoa.

[1] In one of our female rats a tumor growth developed at the site of injection of the spermatozoa, 5 days after 1 cc. of the solution had been introduced under her skin. This grew rapidly for 30 days, without any apparent sepsis, and was then aseptically removed. The rat weighed 158 grams, and the tumor, which was encapsulated, weighed 40.4 grams. On autopsy the rat appeared normal, and eight fetuses of about mid-term were found in the uterus. The growth was diagnosed by Dr. H. G. Wells as fibro-sarcoma.

3. Within limits, the degree of immunity appears to be proportional to the amount of the antigen injected.

4. Immunization with spermatozoa does not affect the sexual cycle (ovulation and rut) of the female (confirming the work of Dittler).

5. Subcutaneous injection of spermatozoa into adult male rats tends to cause destruction of the spermatozoa and atrophy of the testes (confirming the work of De Leslie on rats, and Guyer on rabbits). This effect may be only temporary.

6. Subcutaneous injection of rooster sperm-suspension into egg-laying hens, does not influence the rate of egg production, but renders the eggs infertile for a period of from 12 to 67 days. Injection of chicken liver extract does not produce infertility of the eggs.

BIBLIOGRAPHY

(1) Mechnikoff: Ann. de l'Inst. Pasteur, 1900, xiv, 369.
(2) Landsteiner: Zentralbl. f. Bakt., 1899, xvi, 13; München. med. Wochenschr., 1902, xlix, 473; Wien. klin. Wochenschr., 1901, xiv, 1132.
(3) Taylor: Journ. Biol. Chem., 1908, v, 311.
(4) Dunbar: Zeitschr. f. Immun., 1910, iv, 740.
(5) De Leslie: Cited by Ricketts (6), p. 295.
(6) Ricketts: Infection, immunity and serum therapy, 1911, 296.
(7) Pearce: Journ. Exper. Med., 1914, xix, 277.
(8) Kohlbrugge: Arch. f. Entwicklngsmechn., 1912, xxxv, 165.
(9) Editorial, Journ. Amer. Med. Assoc., 1921, lxxvii, 43.
(10) Waldstein and Ekler: Wien. klin. Wochenschr., 1913, xxvi, 1689.
(11) Metalnikov and Strelnikov: Zeitschr. f. Immun., 1913, xvii, 186.
(12) Herrmann and Stein: Wien. klin. Wochenschr., 1916, xxix, 718.
(13) Kolmer: Infection, immunity and specific therapy, 1920, 538.
(14) Westermark: Hygeia, Stockholm, 1920, lxxxiii, 33.
(15) Dittler: Münch. med. Wochenschr., 1920, lxvii, 1495; Zeitschr. f. Biol., 1920, lii, 72.
(16) Dervieux: Compt. rend. Acad. d. Sci., 1921, clxxii, 1384.
(17) Moore: Journ. Exper. Zoöl., 1921, xxx, 365; Personal communication, July, 1922.
(18) Guyer: Amer. Naturalist, 1922, lv, 637; Journ. Exper. Zoöl., 1922, xxxii, 207.
(19) Hektoen: Journ. Amer. Med. Assoc., 1922, lxxviii, 704.
(20) Donaldson: The rat, 1915, 19.
(21) King and Stotsenburg: Anat. Record, 1915, ix, 403.

STUDIES OF THE THYROID APPARATUS

IX. The Effects of the Loss of the Thyroid and Parathyroid Glands at 100 Days of Age on the Growth in Body Length, Body Weight and Tail Length of Male and Female Albino Rats

FREDERICK S. HAMMETT

From the Wistar Institute of Anatomy and Biology, Philadelphia

Received for publication October 16, 1922

It is well known that a lack of thyroid secretion, whether produced by operative removal or a failure of development of the thyroid gland, causes a retardation of growth, or a loss in body weight in mammals. The more important contributions attesting the general occurrence of this phenomenon are those of Reverdin (1), Schiff (2), von Eiselsberg (3), Hertoghe (4), Gley (5), Moussu (6), Roger and Garnier (7), Lanz (8), Hofmeister (9), de Mira (10), Smith (11), Haushalter and Jeandelize (12), Leonhardt (13), Palmer (14), Tatum (15), Simpson (16), Launoy (17) and Larson (18).

The studies of the effects of the lack of parathyroid secretion have been mainly confined to observations concerning the resultant tetany. The data on the body growth of parathyroidectomized rats are meager. Yet at present the albino rat is the best animal to use for studies of this sort because it is the only laboratory animal for which we have for comparison practically complete standard growth curves of the various organs and of the body as a whole, and because it can survive the loss of the parathyroid glands for long periods.

Erdheim (19) has incidentally reported a loss of weight in parathyroidectomized rats. Iselin (20), in a small series, observed an arrest of growth, while Farner and Klinger (21) state that normal development in their rats was not greatly disturbed by the loss of the parathyroids.

The literature of the studies of the thyroid apparatus is appallingly voluminous, yet I have failed to find therein any attempt to make a comprehensive, systematic investigation of the rôle of the thyroid and

parathyroid glands in the growth of the body as a whole and of its parts, in which differences due to age and sex are included.

Such a study was begun in this laboratory somewhat over two years ago. The present paper is a report and an interpretation of the effects of thyro-parathyroidectomy and parathyroidectomy on the growth in body length, body weight and tail length of male and female albino rats operated at 100 days of age and carried through to dissection when 150 days old.

The age of 100 days was taken as the starting point of one of the periods of study because, as shown by Donaldson's charts (22), rats at this age have about completed their period of rapid growth. The age of 150 days was chosen as the terminal point because at this age the rat is growing at the rate characteristic for the mature animal.

Later communications will take up the changes induced in the growth of the various organs of the body by these processes; the changes in the water content of the brain and spinal cord; the changes in the refractive index of the blood serum and the changes in the chemical composition of the long bones. Similar studies of rats operated at 23, 30, 50, 65 and 75 days are under way. The results will be published as the various series are completed.

Procedure. A detailed description of the selection and care of the rats will be given at this time as illustrative of the general procedure used throughout.

All the rats were from the Experimental Colony of "gentled" animals. It is necessary to use tame, gentle, relaxed rats because of the high mortality after the loss of the parathyroids of the ordinary excitable stock albinos. The reasons for this have been discussed in previous papers (23), (24), (25). Credit for the successful maintenance of such a gentled stock is due Miss F. Louise Duhring, Curator of the Colony, Miss Ruth Meeser, Miss Ida Teller and Miss Elizabeth Justice.

In the beginning the gentled rats were transferred from the colony to the laboratory a few days before the time of operation in order to do away with any possible exciting effects of a sudden change of environment. However, because of the large number of rats necessary, a change was made and each litter of rats with its mother was put into a separate cage in a special room in the colony house a day or two after birth and was then gentled up to the day of operation by Miss Justice.

The litters were weaned at from 23 to 28 days after birth and the sexes were then separated. Each litter was kept in a cage by itself throughout the period of observation. The general plan was to use litters which

contained five or more rats of the same sex. Occasionally, however, litters containing four rats of the same sex were used at the tag end of the series when only one or two more operated rats were needed.

When litters of five rats were used, two were thyro-parathyroidectomized, two were parathyroidectomized and one was kept as the control.

The anatomical relationships of the thyroid and the parathyroid glands makes the removal of the thyroid without the parathyroids almost if not quite impossible. The operative procedure has already been described (23). Thus litters and sex controls were maintained throughout. When litters of six or more were available, one rat was used for testing the effects of the operation as such without the removal of either of the glands. This rat was called the operated control. It was put under deep ether anesthesia, the thyroid was exposed but not removed, the wound was closed and the rat returned to its cage.

The diet was the same as that of the Experimental Colony. It was uniform for all and contained wheat, barley, rice, oats, cornmeal, hominy, peas, beans, lentils, macaroni, fish, meat, liver, milk, eggs, lettuce, spinach, celery, cabbage, carrots, corn and oranges.

The rats were fed once a day and were given water *ad lib.* They were kept in warm, light, well-ventilated rooms in clean well-ventilated cages designed by Dr. Milton J. Greenman. The cages were so constructed that liquids dropped into pans below the bottoms, thus avoiding contamination of the bedding. The bedding was wood wool and was renewed every two weeks or oftener if necessary. No parasites obtained their existence from these rats. Nor did the animals give any evidences of pathological disturbances apart from those induced by the lack of the thyroid and parathyroid secretions.

It can not be too much emphasized that successful experimentation in studies of this sort depends largely upon the care and attention given the animals. They must be kept in a clean, warm, light, well-ventilated environment, plentifully supplied with a varied diet and water, and treated kindly and with gentleness. Rats are just as sensitive as are children to brusque or mechanical handling, sharp and irritating noises or unclean surroundings.

A total of 130 rats was used for the study of the effects of thyroparathyroidectomy and parathyroidectomy at 100 days of age. Owing to incomplete removal of the thyroid or parathyroids or to deaths following operation the final number of animals available for this study was 76. Their distribution is given in table 1. The uniform distribution

of the rats as to sex and operated group is to be noted. The abbreviations—thypars and parathys—as found in the table, will be used from now on instead of the inconvenient expressions, thyro-parathyroidectomized rats and parathyroidectomized rats.

Because of the small number of operated controls available in this series their growth is not specifically considered in comparison with that of the other groups. The general course of the growth of those rats, however, was not significantly different from that of the controls.

Only those values were used in this report which were obtained from thyro-parathyroidectomized rats in which no trace of thyroid tissue was found at dissection; from parathyroidectomized rats in which no parathyroid tissue was found and from their controls.

TABLE 1

The distribution of the rats used for the values reported

	CONTROLS	THYPARS	PARATHYS	TOTALS
Males	11	12	14	37
Females	11	13	15	39
Totals	22	25	29	76

The rats were weighed and the body length and tail length measured at the time of operation and of dissection 50 days later. The body weights were also determined weekly. Only the statistical data of the various measurements are given in this paper. The detailed tabulations of the individual measurements are on file at The Wistar Institute and are available for inspection.

The growth of the male rats. Before taking up the description and discussion of the course of the growth in body weight during the 50-day interval between the time of operation and dissection, the differences in body and tail length and body weight existing between the controls and the two groups of test rats at the beginning and the end of the period of observation will be considered.

In table 2 there are given the means, the standard deviations, the probable errors of the means and the coefficients of variability of the three measurements on the three groups.

Comparing first the initial status it is evident that as far as body and tail length and body weight are concerned, no valid differences obtained between the three groups. Thus there is had for study three groups of

rats of practically the same initial size and comparison between them at later periods can be made without corrections. At 100 days the variability of all factors within the group is somewhat less in the control than in the thypar and parathy groups. The two latter show approximately the same values. The variability in body weight is close to the

TABLE 2

The data on the body length, body weight and tail length of the male control, thyroparathyroidectomized and parathyroidectomized rats at the time of operation (100 days) and of dissection (150 days)

	100 DAYS			150 DAYS		
	Controls	Thypars	Parathys	Controls	Thypars	Parathys
Body length in millimeters						
Mean	180.0	176.1	175.8	202.5	186.5	189.9
Stand. Dev.	10.5	14.6	13.7	5.3	10.5	9.1
P.E.M.	2.1	2.8	2.5	1.1	2.0	1.6
Coef. Var.	5.8	8.3	7.8	2.6	5.6	5.0
Body weight in grams						
Mean	163.8	160.0	156.7	229.8	180.0	192.8
Stand. Dev.	30.6	37.9	37.5	26.3	36.2	33.5
P.E.M.	6.2	7.4	6.8	5.3	7.0	6.0
Coef. Var.	18.7	23.7	23.9	11.4	20.1	17.4
Tail length in millimeters						
Mean	158.6	154.3	155.3	172.2	159.3	163.9
Stand. Dev.	10.9	12.3	12.0	7.0	11.6	10.4
P.E.M.	2.2	2.4	2.0	1.4	2.3	1.9
Coef. Var.	6.9	8.0	7.7	4.1	7.3	6.4

Stand. Dev., standard deviation.
P.E.M., probable error of mean.
Coef. Var., coefficient of variability.

value reported by Jackson (26) and somewhat above that found by King (27). It is much greater than that found in body and tail length. This greater variability in body *weight* at a given age over that in body and tail *length* and the similarity in variability in the two latter is significant.

Now growth may be considered as a result of two different stimuli acting upon the cells of the organism. The first is the stimulus to cell

division. The second is the stimulus to increase in volume of cells by incorporation or deposition of the assimilated foodstuffs. Since the two processes maintained by these two stimuli are not entirely interdependent it is possible to separate the results of their action on the basis of the predominating influence of one or the other. The two processes go hand in hand. The predominance of one or the other is factored by the age of the animal and the available food, to mention only two.

Growth in body length is predominantly due to cell division. Growth in body weight is largely a matter of increase in the size of the cells.

The fact that the variability in body *weight* of these 100 day old rats is greater than the variability in body and tail *length* shows the greater stability of the growth in cell number. Otherwise the scatter in the length values would approximate that of the weight values.

This leads to the idea that the growth in cell volume is first to respond unfavorably to disturbing influences and that the response is the more marked. As far as the studies in which I am now engaged are concerned, such is found to be the case as will be shown presently.

Turning now to a comparison of the absolute values of the thypars and the controls at 150 days of age it is seen that the former are shorter and lighter than the latter. Hence growth in general has been retarded by the combined loss of the thyroid and parathyroid secretions.

Within the group of controls the variability of the three values measured has decreased with time. This indicates a tendency toward eventual uniformity in size and rate of growth—a natural standardization. The variability in body length of the thypars decreased somewhat. Although the variability in body weight and tail length showed a slight decline the degree is insufficient to indicate anything but a general tendency. The decrease in variability in this group was much less than that of the controls. This is to be expected since the general growth was less and perhaps also because of the interruption of the normal progress of events. Nevertheless, the decrease in variability in body length is much greater than that in body weight. This is a demonstration of the principle that the growth in cell number is less susceptible to disturbance than is growth in cell volume.

Comparing the absolute values of the parathys with those of the controls at 150 days it is evident that the loss of the parathyroid secretion has resulted in a retardation of growth in body and tail length and body weight. This was hardly due to a group difference in thyroid size following parathyroidectomy since the mean per cent difference in weight of this organ from that of the controls was but −4.4 with a probable error of the mean of 1.9.

In table 3 is given the absolute per cent increment on age in body and tail length and body weight of the three groups. In order to obtain a satisfactory measure of the relative retardation in growth due to the experimental procedures the relative increments of the thypars and parathys have been calculated using the per cent increment of the controls as the base. The values are given in table 4.

TABLE 3

The absolute per cent increments in body length, body weight and tail length of the male rats during the 50 day period

	CONTROLS PER CENT INCREMENT	THYPARS PER CENT INCREMENT	PARATHYS PER CENT INCREMENT
Body length	12.5	5.9	8.0
Body weight	40.3	12.5	23.0
Tail length	8.6	3.2	5.5

A study of this tabulation brings out the following facts:

1. The combined loss of the thyroid and parathyroid glands retards growth in body length, body weight and tail length to a much greater degree than does the loss of the parathyroids alone.

2. Thyro-parathyroidectomy retards growth in body weight (cell volume) more than it does growth in body length or tail length (cell number). This is a further demonstration that the growth in cell number is less susceptible to interfering factors than is the growth in cell volume.

TABLE 4

The relative growth of the male controls, thypars and parathys

	BODY LENGTH	BODY WEIGHT	TAIL LENGTH
	per cent	*per cent*	*per cent*
Controls	100.0	100.0	100.0
Thypars	47.2	31.0	37.2
Parathys	64.0	57.1	63.9

3. The same is true but to a lesser extent after parathyroidectomy. The brake that has here been put on the growth processes has had an almost equal influence on the three factors measured. This is indicative of a fundamentally different cause of the retardation from that following thyro-parathyroidectomy. The analysis of this difference will be made shortly.

4. The retardation in tail length after the loss of the thyroid is greater than the retardation in body length growth. From this fact there develops the principle of the forwarding of essential growth. This means that there is an inherent mechanism which tends to protect the growth of essential structures when a diminution of the stimulus to grow occurs, while the growth of relatively unessential structures is allowed to lapse. This forwarding of essential growth in times of emergency is similar to the tendency for the conservation of essential organs in starvation. The resistance of the central nervous system to loss during inanition is well known from the studies of Donaldson (28)

TABLE 5

Summary of the weights of the three groups of males at each period of measurement

	DAY							
	100	107	114	121	128	135	142	150
Controls								
Mean	163.8	181.5	190.9	199.8	207.2	215.0	221.6	229.8
Stand. Dev.	30.5	30.9	24.4	22.6	23.0	23.5	24.2	26.3
P.E.M.	6.2	6.6	5.0	4.6	4.9	4.8	4.9	5.3
Thypars								
Mean	160.0	159.6	161.1	163.0	165.6	170.6	175.2	180.0
Stand. Dev.	37.5	38.7	41.5	39.5	37.4	38.1	38.2	36.2
P.E.M.	7.4	7.5	8.1	7.7	7.3	7.4	7.4	7.0
Parathys								
Mean	156.7	150.8	158.3	162.3	166.9	177.6	181.7	192.8
Stand. Dev.	37.5	33.3	29.9	28.4	32.5	35.7	35.7	33.5
P.E.M.	6.8	6.5	5.4	5.1	5.9	6.4	6.4	6.0

and Hatai (29). The connection between the growth of the central nervous system and body length and tail length in my experiments will be reserved for a later paper. At this time I merely wish to point out the principle of the forwarding of essential growth, without going into an extensive analysis of the phenomenon.

It might be concluded from the foregoing that the parathyroid glands are growth-promoting organs since by their absence a retardation of growth is produced. Such is not the case.

In chart 1 there are given the curves of the absolute weights of the

three groups during the interval from 100 to 150 days as measured weekly. The data from which the curves are drawn are given in table 5.

On account of the large number of rats being used and in order to enable other work to be done without interruption, Tuesdays and Saturdays were set aside as days on which the rats are weighed. Since, however, rats come to be 100 days of age on other days than Tuesdays and Saturdays the ages at weighing after the operations were not always

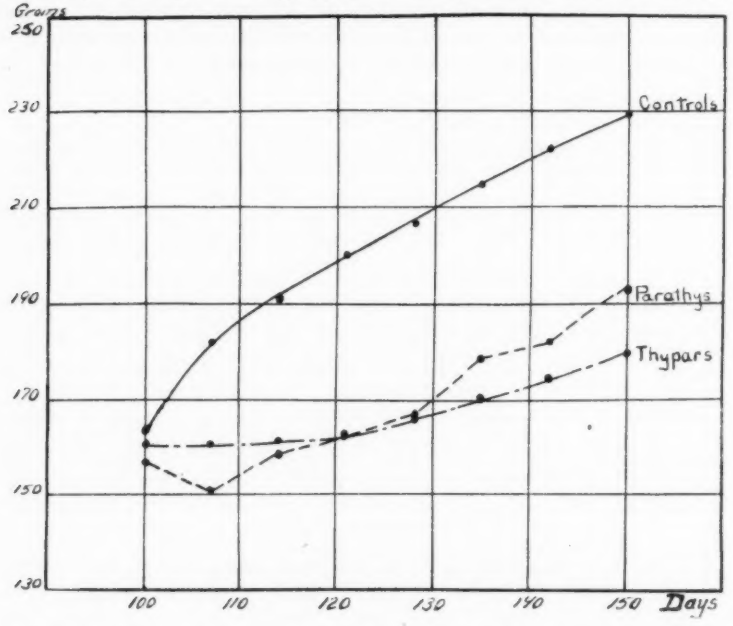


Chart 1. The growth in grams on age of the male control, thypar and parathy groups.

107, 114, 121, 128, 135 and 142 days. Sometimes they were a day or two more or less. Nevertheless, once the weighings were begun they were made every seven days up to the day of dissection. In order to get the weights of each group on to a common age basis use was made of the age-weight table in Donaldson's book on *The Rat* (22) according to the following example.

The litter of male rats designated by the letter R was 100 days old on a Wednesday. They were therefore operated and measured on that

day. The next weighing day for this set was the following Tuesday when the rats were 106 days old. Rat R-5 weighed 198 grams on this day. But the age basis for measurement is 107 days. Therefore, we refer to table 74, page 151 in the above cited work and find that a rat which weighs 198 grams on one day should weigh 199 grams the next day. Hence 199 grams is taken as the weight of rat R-5 when 107 days old. When the rats were older than the common age basis on the day of weighing subtraction of the appropriate value was done. There can

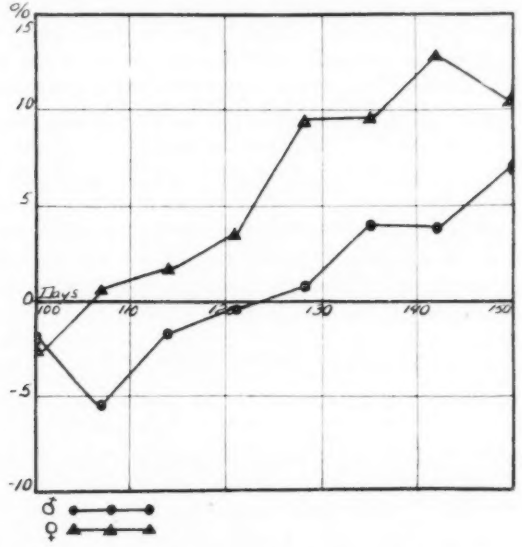


Chart 2. The absolute per cent differences of the weights of the parathyroidectomized from the thyro-parathyroidectomized rats on age.

be no serious objection to this procedure since litter controls were always used and since the corrections were made on the basis of well-established standards. Moreover, it may be noted here that after operation all of the thypars were consistently lower in weight than their individual controls and that but one of the 14 parathys showed a weight above its control.

From table 5 it is evident that the continuously lower weights of the operated groups is statistically valid. From the fact that the differences between the thypars and the parathys were at no time statis-

tically valid it might be inferred that the retardation was due to the parathyroid loss and not to thyroid removal. Nevertheless, it can be seen from chart 1 that there is a well-defined tendency for the growth of the parathys to be increasingly superior to that of the thypars. When the per cent differences in weight of the parathys from the thypars are calculated and plotted as in chart 2 this tendency becomes the more clear. The curves for this relation in the operated groups of both sexes are given. However, for valid quantitative comparison further analysis is necessary.

TABLE 6

The growth capacity of the male control, thypar and parathy groups from 100 to 142 days of age at intervals of seven days

	INTERVAL						
	100–107	107–114	114–121	121–128	128–135	135–142	142–150
The observed values							
Controls	10.0	5.2	4.7	3.7	3.8	3.0	3.2
Thypars	−0.3	0.9	1.2	1.6	3.0	2.7	2.4
Parathys	−3.8	5.0	2.5	2.8	6.4	2.3	5.4
The expected values							
Controls	5.9	4.5	3.9	3.4	2.9	2.6	2.3
Thypars	6.3	6.3	6.1	6.0	5.7	5.3	5.0
Parathys	6.6	7.2	6.4	6.0	5.6	4.8	4.5
The observed minus the expected values							
Controls	4.1	0.7	0.8	0.3	0.9	0.4	0.9
Thypars	−6.9	−5.4	−4.9	−4.4	−2.7	−2.6	−2.6
Parathys	−10.4	−2.2	−3.9	−3.2	−0.8	−2.5	−0.9

An index of the ability of the growing organism to add on weight to itself is given by the increment of weight per 100 grams of body weight per unit of time. This value will be called the growth capacity and will be represented by the abbreviation G. C. In these studies the G. C. is obtained by dividing the grams increment for each week by the weight at the beginning of the week and multiplying by 100.

The results of this calculation for the three groups are given in table 6 as the observed G. C. Since the period 142–150 days is one of eight days instead of seven the value recorded is seven-eighths of the

original calculated. From table 6 it is plain that the combined loss of the thyroid and parathyroid glands has produced a definite reduction of the G. C. below that of the controls. The loss of the parathyroids has produced a similar but less constant effect.

A direct comparison of the observed G. C. of the three groups does not yield a valid quantitative index of the relative growth because the thypars and parathys are much less heavy than the controls. They would then be expected to have a greater G. C. than the controls, since the albino rat, like other animals, adds to itself regularly decreasing amounts of body tissue as it grows older (which is to say heavier). In other words, the G. C. diminishes with increasing age and weight in the normal albino rat.

Now the normal growth of the albino rat has been determined and the values tabulated by Donaldson (22). These values provide a standard basis for comparison of two or more groups of rats under observation. From them can be calculated the expected G. C. for rats of any weight during any given interval (within normal limits, of course). From the foregoing it is plain that when the same basis for comparison is used for the controls as for the test rats, and when all the rats are from the same stock and are maintained under the same conditions of environment and diet throughout, the inter-group comparison of the deviations of the observed G. C. from the expected gives a satisfactory measurements of the effects of the experimental procedure upon the ability to grow in weight.

The method of calculating the expected G. C. from the observed weights is as follows. From table 5 we find that the mean weight of the control group at 114 days was 190.0 grams. From table 74 in Donaldson's book (22) it is seen that a rat of this weight should weigh 198.3 grams 7 days later. This increment of 7.4 grams divided by the initial weight of 190.0 grams times 100 gives the G. C. of 3.9. That is to say, a rat of 190.0 grams weight on a given day should add on 3.9 grams of weight per 100 grams of initial body weight during the next 7 days. However, from table 5 it is seen that the observed weight after 7 days, or at 121 days, was 199.8 grams, an increment of 8.9 grams or a G. C. of 4.7. Hence the control rats were adding on more weight per unit weight per unit time at this period than was to be expected.

The expected G. C.'s for the three groups are tabulated in table 6, together with the differences of the expected from the observed. I will use the graphs in charts 3 and 4 as the basis of the discussion.

Comparing first the curves for the observed and the expected G. C.

of the controls—the curve for the observed G. C. always lies above that of the expected and from 107 days on is roughly parallel thereto. This shows that the relative rate of growth of the group with respect to the actual body weight was the same as that of the standard, but that it was on a somewhat higher level.

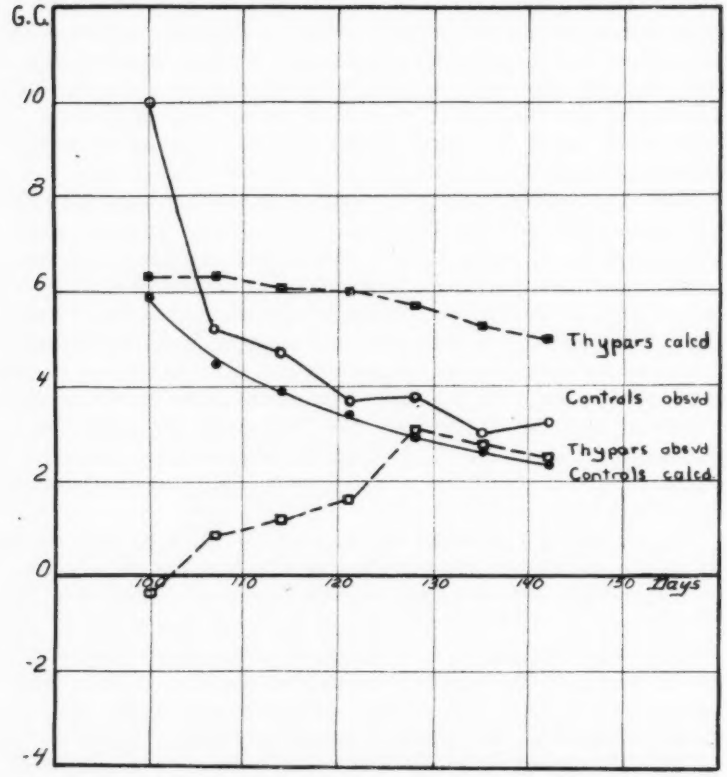


Chart 3. The observed and expected growth capacities of the male control and thypar groups.

When the curves for the observed and the expected G. C. of the thypar group (chart 3) are compared a different relation is found.

The curve of the observed is always well below that of the expected. The ability to grow was absolutely stopped for the first week. From

then on until the 128th day the G. C. increases and then slowly decreases parallel to the expected and the standardized controls, but at a much lower level than normal for the actual body weight.

In order to simplify the presentation at this stage it will be assumed that the growth disturbances following thyro-parathyroidectomy are predominantly due to the loss of the thyroid gland and that the simultaneous loss of the parathyroids has had but a minor effect. This assumption is partially justified by the findings published in the two preceding papers of this series (30), (31).

Now the course of the curve of the G. C. after the removal of the thyroid shows a reassumption by the organism of its ability to grow which was completely stopped in the beginning. This renewal of growth and gradual increase in the capacity to grow is an expression of an attempt to reach a state of equilibrium between the growth impulse and the diminution of the stimulus to growth caused by the loss of the thyroid. This attempt was successful as shown by the parallelism of the observed thypar and the standardized control G. C. curves from the 128th day on.

It is obvious that the promotion of growth in many living organisms is not dependent on the products of the glands of internal secretion as known in the higher animal forms. At present, therefore, we are not justified in attributing all growth in those organisms which possess glands of internal secretion to the participation of the organs which are known specifically to forward growth. Now the resumption of growth after the loss of the thyroid by these rats may be due either to the tendency inherent in all living things or it may be due to the supplying of a growth-promoting stimulus in increased amounts from some secondary particular source such as the hypophysis. The fact that the hypophysis is known to enlarge after the loss of the thyroid secretion would support this idea if such enlargement means an increased functional activity. This does not necessarily imply a vicarious functioning of the hypophysis for the thyroid. Such can not be because the evidence clearly shows that the thyroid has a peculiar and specific regulative function in metabolism which is not possessed by the hypophysis.

Nevertheless, it is plain that in time of emergency, when the tendency to grow is obstructed because of the lack of an essential stimulus from the thyroid, there is an attempt by the organism to supply the necessary amount of growth stimulus by an increase in the activity of some secondary growth-promoting mechanism.

The process can be pictured as follows.

In the normal, sexually mature, growing male rat the predominant stimulus to growth in cell volume is supplied by the thyroid. Other stimuli may exist but their action is overshadowed by the latter. This is shown by the cessation of growth immediately after the loss of the thyroid. In view of the studies of Kendall (32) and Plummer and Boothby (33) on the effect of the administration of thyroxin in cretinism and adult myxedema it appears that through the regulatory influence of this compound on metabolism it acts as a stimulus to such growth. On removal of the thyroid, growth is stopped because of the abrupt deprivation of this primary stimulus. But since the G. C. begins to increase shortly thereafter it is evident that a new source of supply of a stimulus to growth (secondary in nature) is being called upon with favorable response.

Because of the enlargement of the hypophysis after thyroidectomy it is possible that this gland is furnishing the desired stimulus in connection with the inherent ability to grow by accretion, not as a new function but as an increase in a function already possessed and possibly dormant at this age. But this reserve stimulus fails to replace completely the stimulus provided by the thyroid, otherwise the G. C. would rise to the level of the expected instead of remaining consistently below. This failure arises because the secondary stimulus does not induce the same degree of metabolic activity. Therefore the point where the curve of the G. C. of the thypar group stops rising and begins to fall parallel with that of the standardized controls, indicating a relatively equal rate of growth per unit of body weight, is the point where the secondary growth-promoting stimulus has reached its maximum effect consistent with the lowered metabolism due to the lack of the thyroid secretion. From then on growth proceeds at a lower level but in the same fashion as if it were at the normal level.

According to this interpretation the thyroid is the primary source of the stimulus to growth in cell volume shortly after sexual maturity. Its growth-promoting properties are confined to its stimulating action on metabolism. When the thyroid function is lacking metabolism may go on but at a lower level than to be expected from the actual body weight. Hence exogenous growth may go on but at a much lower level than that expected from the body weight. Although other secondary sources of stimuli to growth may be called upon they are insufficient to raise the G. C. to the level normal for the actual weight because of the lowered metabolism due to the loss of the thyroid secretion.

Comparing the curve of the observed with the expected G. C. of the

parathy group (chart 4) we find that the latter pursues a fairly regular course while that of the former is highly irregular and twice reaches the expected values. During the first week the ability to grow was not only stopped but weight was lost. At no time thereafter, however, was

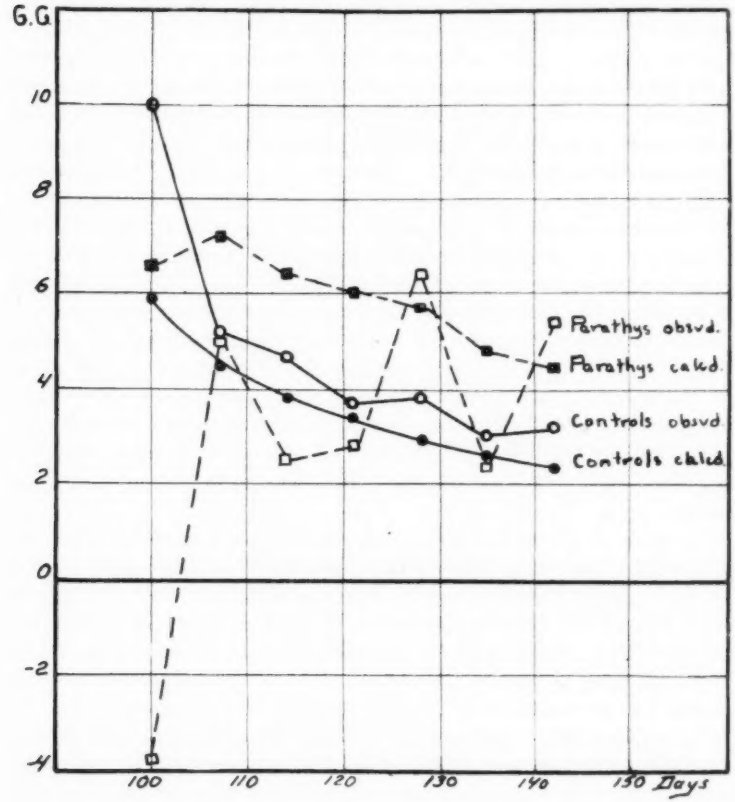


Chart 4. The observed and expected growth capacities of the male control and parathy groups.

growth capacity lacking. This loss of weight during the first week after operation is due to the effects of acute parathyroid tetany, which is more marked immediately after the loss of the parathyroid secretion than at any time thereafter. The fact that the largest number of rats

which die of acute parathyroid tetany do so during the first 48 hours after parathyroidectomy testifies to the validity of this interpretation.

The irregularity of the curve of the observed G. C. consists in an alternate marked increase and decrease with an increasing tendency for an approach to the expected. During the fifth and seventh weeks the observed G. C. is slightly greater than the expected. This fact shows that the ability to grow *per se* has neither been destroyed nor quantitatively diminished. Since the G. C. of the first, third, fourth and sixth weeks, however, was lower than the expected on the basis of the actual body weight, it is evident that the expression of this ability to grow has been markedly interfered with. There are two possible interpretations of this interference. Either the toxemia produced by the loss of the parathyroid secretion so poisons the cells that growth in cell volume is retarded, or the tetany due to the toxemia burns up so much of the assimilated food that but little is available for the formation of additional tissue. A combination of these two processes may well occur.

Whichever may be the actual cause it would appear as if the periods of decreased G. C. followed by periods of increased and normal G. C. are the results of an accumulation within the organism of the toxic products due to the loss of the parathyroids which directly or indirectly retard the expression of the ability to grow, and their subsequent destruction or elimination with the release to that G. C. normal for the weight.

The basis of the retardation in growth following parathyroidectomy is therefore quite different from that occasioned by thyro-parathyroidectomy. The retardation of growth following parathyroidectomy is not a destruction of or a diminution in the ability or the stimulus to grow, but is a poisoning of the organism so that the expression of the effects of these factors is obstructed. The normal potential G. C. is present but prevented from exerting its effect except occasionally. On the other hand the retardation of growth following thyro-parathyroidectomy is not a poisoning of the organism but is an actual diminution in the ability to grow due to the loss of a major stimulus to growth and metabolism.

The parathyroid loss is of relatively minor importance in the growth retardation of the thypar group as shown by the superior G. C. of the parathyroidectomized rats; by the sharp irregularity of the curve for their G. C.; by the occurrence of periods of G. C. normal for their body weight; by the fact, already published (30), that disturbances in calcium metabolism as shown by dental defects frequently occur after para-

thyroidectomy and but rarely after thyro-parathyroidectomy; and by the fact that the mean per cent variability in body weight from week to week of the parathys was 3.70, while that of the thypars was but 0.65 and that of the controls 1.20. The low value of the thypars as compared with that of the controls indicates that the removal of the thyroid and

TABLE 7

Data on the body length, body weight and tail length of the female control, thyro-parathyroidectomized and parathyroidectomized rats at the time of operation (100 days) and of dissection (150 days)

	100 DAYS			150 DAYS		
	Controls	Thypars	Parathys	Controls	Thypars	Parathys
Body length in millimeters						
Mean	171.5	171.5	168.9	190.2	176.0	177.5
Stand. Dev.	7.5	9.1	11.7	6.5	7.7	10.6
P.E.M.	1.5	1.7	2.0	1.3	1.4	1.8
Coeff. Var.	4.4	5.3	6.9	3.4	4.4	5.9
Body weight in grams						
Mean	141.3	140.8	137.0	177.0	130.8	144.1
Stand. Dev.	19.3	19.6	27.3	21.2	22.7	24.5
P.E.M.	3.9	3.7	4.9	4.3	4.2	4.4
Coeff. Var.	13.7	13.9	19.9	12.0	17.3	17.0
Tail length in millimeters						
Mean	153.3	154.5	152.3	163.5	156.6	157.3
Stand. Dev.	6.0	5.7	10.7	6.2	6.0	10.3
P.E.M.	1.2	1.1	1.9	1.3	1.1	1.8
Coeff. Var.	3.9	3.7	7.0	3.8	3.8	6.5

parathyroids has reduced normal fluctuations. This I take to be because of the lowered metabolism due to the loss of the thyroid. The high value of the parathys as compared with the controls indicates that the loss of these glands alone has introduced a factor for variability in body weight, directly the opposite effect of that produced by thyro-parathyroidectomy. The very low value of the thypars as compared with that of the parathys indicates that the variability producing factor of the latter, which I take to be the factor retarding the expression of growth capacity, is quite effectually neutralized.

The growth of the female rats. In table 7 there are given the means,

the standard deviations, the probable errors of the means and the coefficients of variability of the body length, body weight and tail length of the control, thypar and parathy groups of females at 100 and 150 days of age. It is clear that the initial absolute status of the three groups was the same with respect to the three factors measured and hence the extent of the changes induced by the experimental procedures is comparable without correction with the changes which took place in the controls.

There were no consistent inter-group differences in variability within the group at 100 days. The variability in body weight was much greater in all the groups than that in body or tail length. This supports the theory of growth developed from similar findings with the males.

From the figures recorded under the 150 day section it is obvious that, as with the males, growth in general was retarded both by the combined loss of the thyroid and parathyroid glands and by the loss of the parathyroids alone. The changes in variability from those obtaining at 100 days were not sufficiently consistent to warrant an attempt at interpretation. It may be noted, however, that the relatively higher variability in body weight was maintained. The mean per cent difference in weight of the thyroid of the parathy females from that of the controls was -8.2 with a probable error of the mean of 1.5.

At 100 days the females were in general shorter and lighter than the males. The tail length differences were negligible. The variability within the group was less than that of the males. The same held true for the female controls at 150 days.

In table 8 the absolute per cent increment on age in body and tail length and body weight of the three groups is recorded. Using the per cent increments of the controls as the base the relative increments were calculated and are given in table 9. A study of this table yields the following:

1. The combined loss of the thyroid and parathyroid glands retards growth in body length, body weight and tail length more than does the loss of the parathyroids alone. The body weight actually decreases as a result of thyro-parathyroidectomy.

2. Thyro-parathyroidectomy retards growth in body weight more than it does growth in body length or tail length.

3. The same is true for parathyroidectomy. The foregoing, and the fact that both with the males and with the females the growth retardation of body length and tail length after parathyroidectomy is approximately the same and much less than that of body weight is further support for the belief that the growth in cell number is less susceptible to interfering factors than is growth in cell volume.

4. The retardation in tail length growth after thyro-parathyroidectomy is slightly greater than the retardation of body length growth. This supports the theory of forwarding of the growth of essential structures developed from similar findings with the males.

TABLE 8

The absolute per cent increments in body length, body weight and tail length of the female rats during the 59 day interval

	CONTROLS PER CENT INCREMENT	THYPARS PER CENT INCREMENT	PARATHYS PER CENT INCREMENT
Body length	11.0	2.7	5.2
Body weight	25.1	−7.1	5.2
Tail length	6.7	1.3	3.3

TABLE 9

The relative growth of the female control, thypars and parathys

	BODY LENGTH	BODY WEIGHT	TAIL LENGTH
	per cent	*per cent*	*per cent*
Controls	100.0	100.0	100.0
Thypars	24.5	−28.2	19.4
Parathys	47.2	20.7	49.3

TABLE 10

The differences between the relative growth of the males and females; derived by subtracting the values for the females from table 9 from the similar values for the males in table 4

	BODY LENGTH	BODY WEIGHT	TAIL LENGTH
Thypars	22.7	59.2	17.8
Parathys	16.8	36.4	14.6

In table 10 are given the differences between the relative growth of the males and the females of the operated groups as compared with their respective controls. This comparison shows that the general growth of both groups of operated males was less retarded than that of the females. The difference is more marked in the case of the thypars and the body weight values.

In chart 5 are given the curves of the absolute weights of the three groups during the interval from 100 to 150 days as measured weekly. The data from which these curves are drawn are given in table 11.

From the table it is evident that both the thypars and the parathys remained consistently lower in body weight than the controls after the first measurements. Here, as with the males, the absolute differences between the thypars and the parathys are not statistically valid. Yet from chart 5 it is evident that there was a well-defined tendency for the growth of the parathys to be increasingly superior to that of the thypars. (The latter never regained their initial weight.) When the per cent differences between the parathys and thypars are plotted as in chart 2, this tendency becomes more clear. It is quite similar to that found with the males though more pronounced.

TABLE 11

Summary of the weights of the three groups of females at each period of measurement

	DAY							
	100	107	114	121	128	135	142	150
Controls								
Mean	141.5	151.6	158.8	161.8	167.3	169.3	173.0	177.0
Stand. Dev.	19.3	19.5	21.1	22.3	22.7	23.4	23.5	21.2
P.E.M.	3.9	4.0	4.3	4.5	4.6	4.8	4.8	4.3
Thypars								
Mean	140.8	132.8	132.3	131.0	129.0	129.3	128.8	130.8
Stand. Dev.	19.6	17.6	20.7	23.4	24.9	24.0	24.4	22.7
P.E.M.	3.7	3.3	3.9	4.4	4.7	4.6	4.6	4.2
Parathys								
Mean	137.0	133.5	134.3	135.3	140.9	141.3	145.4	144.1
Stand. Dev.	27.3	26.5	27.7	27.2	26.7	25.6	24.1	24.5
P.E.M.	4.9	4.7	5.0	4.9	4.8	4.6	4.3	4.4

Turning now to the G. C. as a more serviceable index of the effect of the experimental procedures on growth there is recorded in table 12 the observed and calculated expected G. Cs. for the three groups and the values for the observed, minus the expected. The curves for the observed and the expected G. C. values are plotted in charts 6 and 7.

Comparing first the curve for the observed and the expected G. C. of the controls, the curve for the observed G. C. is below that of the expected. This indicates a lower level of growth capacity than was to be expected from the absolute body weights.

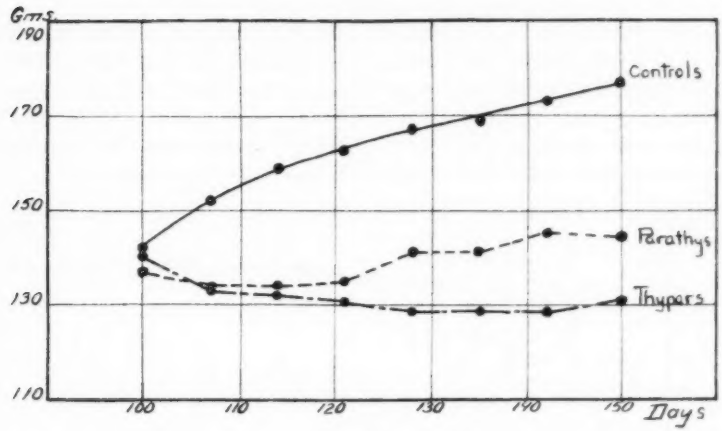


Chart 5. The growth in grams on age of the female control, thypar and parathy groups.

TABLE 12

The growth capacity of the female control, thypar and parathy groups from 100 to 142 days of age at intervals of seven days

	INTERVAL						
	100–107	107–114	114–121	121–128	128–135	135–142	142–150
The observed values							
Controls	7.1	4.8	1.9	3.4	1.2	2.2	2.0
Thypars	−5.7	−0.4	−1.0	−1.5	−0.2	−0.4	1.4
Parathys	−2.6	−0.6	0.7	4.1	0.3	2.9	−0.8
The expected values							
Controls	5.4	4.5	3.9	3.6	3.2	3.1	2.9
Thypars	5.5	6.2	6.4	6.6	6.8	6.8	6.9
Parathys	5.8	6.3	6.2	6.1	5.5	5.4	5.0
The observed minus the expected values							
Controls	1.7	0.3	−2.0	−0.2	−2.0	−0.9	−0.9
Thypars	−11.2	−6.6	−7.0	−8.1	−7.0	−7.2	−5.5
Parathys	−8.4	−6.9	−5.5	−2.0	−5.0	−2.5	−5.8

Although the curve is quite irregular its course is roughly parallel to that of the standardized controls and the course of growth accordingly was close to that to be expected, but on a lower level. The cause of the irregularity is at present inexplicable. As compared with the G. C. of the male controls it is evident that the female controls had both absolutely and relatively a lesser G. C. than had the males.

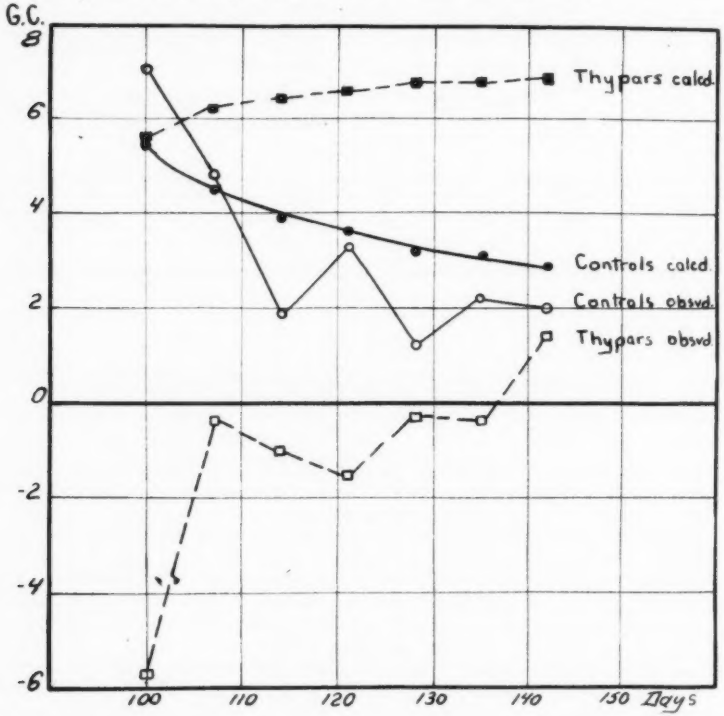


Chart 6. The observed and expected growth capacities of the female control and thypar groups.

When the curves of the observed and the expected G. C. of the thypars are compared (chart 6) it is seen that the observed values are consistently much lower than those of the expected. From the fact that the G. C. is so markedly decreased and negative in value, indicating a loss in weight, immediately following the loss of the thyroid, and remains negative until the 142nd day, it is evident that the deprivation of the

thyroid secretion has diminished the ability to grow to a much greater extent in the females than in the males, for in the latter the G. C. is positive after the first week. However, the direction of the curve of the G. C. of the female thypars shows a striving for recovery which was finally reached and growth resumed at the 142nd day. Nevertheless, growth even at this time was on a lower level than would be

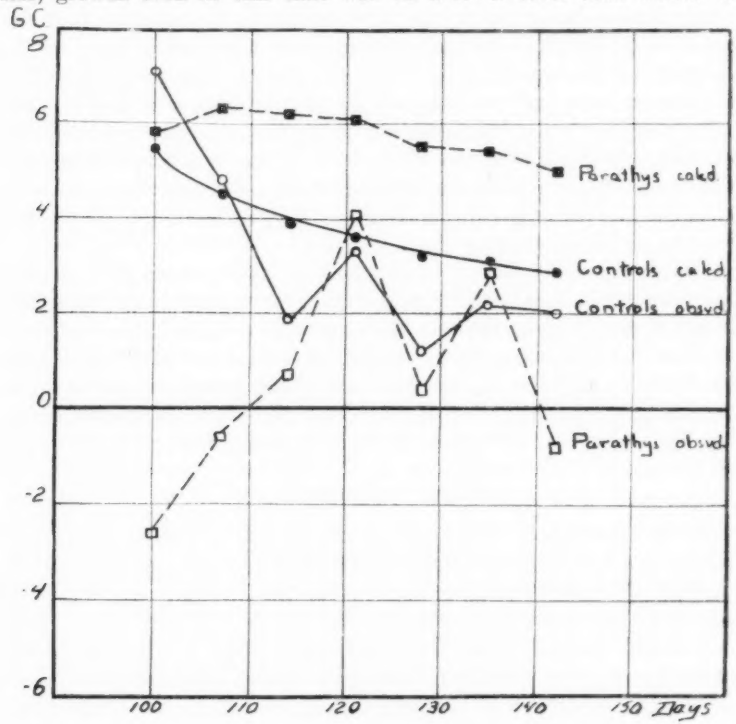


Chart 7. The observed and expected growth capacities of the female control and parathy groups.

expected from the actual body weight. This delay in resumption of growth by the females as compared with the males is not surprising since the former had initially a lesser G. C. than the latter and since the immediate effect of thyroid loss was much more marked. However, the course of the curve of the G. C. of the female thypars is roughly qualitatively like that of the males. The factor for growth retardation

is the same in both sexes although quantitatively different in the intensity of its effect.

Comparing the curve of the observed with that of the expected G. C. of the parathy (chart 7) group it is seen that the observed values always lie below those of the expected, never reaching the latter as they do in the male parathys. This delay as compared with the males in the attainment of periods when the G. C. is that to be expected from the actual body weight is explicable on the same basis as the delay in the recovery of the female thypars. The irregularity and general shape of the parathy curve of the females is the same as that of the males. The causative factor was the same for each although more intense in its growth obstructing effect in the females. In the females as in the males the mean per cent variability in body weight from week to week was higher (2.72) than that of the controls (1.69) or the thypars (1.61) and is to be similarly interpreted.

The general conclusion to be drawn from these observations is that the retardation of growth in body length, body weight and tail length of the females due to the loss of the thyroid or parathyroid glands is qualitatively the same, but quantitatively more intense than is that of the males in all respects, and that the general interpretations based on the changes induced in the males by the experimental procedures are equally applicable to the changes similarly induced in the females.

SUMMARY AND CONCLUSIONS

An examination of the effects on the growth in body length, body weight and tail length of male and female albino rats deprived of the thyroid and parathyroid, or parathyroid glands alone at 100 days of age and allowed to grow until 150 days of age has been made. Both males and females responded qualitatively alike to the loss of the functions of these glands of internal secretion. The response was a retardation in growth of the parts measured. From the quantitative standpoint the growth of the females was retarded or obstructed to a greater degree by the experimental procedures than was that of the males. It would appear from this that the female rat is more dependent upon the stabilizing influence of the thyroid and parathyroid glands in growth than are the males.

Growth in body weight was inhibited to a greater extent than was growth in body length and tail length after the loss of either the parathyroids alone or the thyroid and parathyroids combined. This is taken to indicate that the cell growth by accretion is more susceptible to disturbing factors than is growth by cell division.

Growth in tail length was retarded to a greater degree than was growth in body length after thyro-parathyroidectomy. This is considered as a demonstration that there is inherent in the organism a mechanism by which the growth of essential structures is forwarded in times of emergency, while growth of unessential structures is allowed to lapse.

The retardation in growth due to the loss of the parathyroids was somewhat less than that due to the loss of the thyroid.

The growth capacity of the parathyroidectomized rats showed alternate periods of marked decrease and increase in value, at times reaching the value to be expected on the basis of the observed body weight. This type of irregularity indicates the repeated accumulation and subsequent destruction or elimination of toxic products and a consequent retardation and release of the ability to grow.

The growth capacity of the thyroidless rats does not exhibit such changes. It tends to gradually increase in value from the original low figure obtaining directly after the loss of the thyroid until it reaches a state of equilibrium consistent with the lowered metabolism due to the thyroid removal. Growth then proceeds as usual but at a much lower level than that normal for the actual body weight.

The basis of the growth retardation after parathyroidectomy is therefore quite different from that occasioned by the loss of the thyroid. The former is not a destruction or a diminution in the ability or the stimulus to grow, but is a poisoning of the organism so that the expression of the effects of these factors is obstructed. The normal potential growth capacity is present but prevented from exerting its effect but occasionally. On the other hand the retardation of growth following thyroid removal is not a poisoning of the organism, in the sense used above, but is an actual diminution in the ability to grow due to the loss of a major stimulus to growth and metabolism.

BIBLIOGRAPHY

(1) Reverdin: Rev. med. de la Suisse Rom., 1882, ii, 539.
(2) Schiff: Arch. f. exper. path. u. pharm., 1884, xviii, 66.
(3) v. Eiselsberg: Arch. f. klin. chir., 1895, xlix, 207.
(4) Hertoghe: Die Rolle der Schilddrüse—etc., München, 1900.
(5) Gley: Arch. physiol. norm. et path., 1892, 5s., iv, 391.
(6) Moussu: Compt. Rend. Soc. Biol., 1897, 10s., iv, 82.
(7) Roger and Garnier: Compt. Rend. Soc. Biol., 1901, liii, 1129.
(8) Lanz: Beitr. z. klin. chir., 1905, xlv, 208.
(9) Hofmeister: Beitr. z. klin. chir., 1894, xi, 441.
(10) de Mira: Journ. de physiol. path. gen., 1920, xviii, 976.

(11) SMITH: Journ. Physiol., 1894, xvi, 378.
(12) HAUSHALTER AND JEANDELIZE: Compt. Rend. Soc. Biol., 1902, liv, 600.
(13) LEONHARDT: Virchow's Arch., 1897, cxlix, 341.
(14) PALMER: This Journal, 1914, xlii, 572.
(15) TATUM: Journ. Exper. Med., 1913, xvii, 636.
(16) SIMPSON: Quart. Journ. Exper. Physiol., 1913, vi, 119.
(17) LAUNOY: L'appareil Thymo-thyroidien. These de Paris, 1914.
(18) LARSON: This Journal, 1919, xlix, 55; 1920, liii, 89.
(19) ERDHEIM: Mitt. a. d. Grenzgeb. f. med. u. chir., 1906, xvi, 631.
(20) ISELIN: Deutsch. Zeitschr. f. klin. Med., 1908, xciii, 494.
(21) FARNER AND KLINGER: Mitt. a. d. Grenzgeb. f. med. u. chir., 1920, xxxiv, 354.
(22) DONALDSON: The rat. Memoirs of The Wistar Inst., no. 6, 1915, Philadelphia.
(23) HAMMETT: This Journal, 1921, lvi, 196.
(24) HAMMETT: Endocrinol., 1922, vi, 221.
(25) HAMMETT: Journ. Comp. Psychol., 1921, i, 489.
(26) JACKSON: Amer. Journ. Anat., 1913, xv, 1.
(27) KING: Anat. Rec., 1915, ix, 751.
(28) DONALDSON: Journ. Comp. Neurol., 1911, xxi, 139.
(29) HATAI: This Journal, 1904, xiv, 116.
(30) HAMMETT: This Journal, 1922, lxii, 197.
(31) HAMMETT: Amer. Journ. Anat., 1922, xxxi, 103.
(32) KENDALL: Endocrinol., 1919, iii, 156.
(33) PLUMMER AND BOOTHBY: This Journal, 1921, lv, 295.

CHANGES IN ORGAN WEIGHTS OF THE GUINEA PIG DURING EXPERIMENTAL SCURVY

D. H. BESSESEN

From the Department of Anatomy, University of Minnesota, Minneapolis

Received for publication October 17, 1922

McCarrison and others have observed a remarkable enlargement of the suprarenal glands in the guinea pig during experimental scurvy. The object of the present investigation is to study more carefully the extent of this enlargement in the various stages of scurvy, and to ascertain whether there are also changes in the weights of the other viscera. The work was done under the direction of Prof. C. M. Jackson, to whom I am indebted for advice and assistance.

For the animals used in this investigation, I am also obliged to Prof. R. Adams Dutcher, formerly in the Department of Agricultural Biochemistry of the University of Minnesota, who fed the guinea pigs in his laboratory. The scorbutic diet used was oats and water alone for the first fifteen animals; autoclaved milk was added to the diet for the remaining eleven. The curative diet was orange juice and green feed, with one exception in which boiled rhubarb juice was substituted for the orange juice. After the cure was apparently well established, the green feed alone was used. The twenty-six guinea pigs used are grouped as follows (see table 1):

Group I. Three animals, fed scorbutic diet for 5 days, and found apparently normal at autopsy.

Group II. Three animals, fed scorbutic diet for 10 days; appeared normal at autopsy, excepting a slight intestinal hyperemia.

Group III. Three animals, fed scorbutic diet for 15 days; appeared normal at autopsy, excepting slight intestinal hyperemia and slight congestion of the lungs.

Group IV. Two animals were fed scorbutic diet for 19 days. The autopsies showed a mild degree of scurvy. There were swellings of the costochondral junctions, ecchymoses into the muscles of the pectoral girdle, swelling and hemorrhage around the knee joint.

Group V. Eight animals were fed on scorbutic diet and died in 21

TABLE 1

Body weights of guinea pigs used

PIG NUMBER	SEX	MAXIMUM BODY WEIGHT IN GRAMS	FINAL BODY WEIGHT IN GRAMS	DIFFERENCE IN GRAMS	DIFFERENCE IN PER CENT	AVERAGE PER CENT DIFFERENCE FOR GROUP
Group I. 5 days on scurvy diet						
71	M	193	180.9	−12.1	−6.30	−5.8
70	M	244	227.5	−16.5	−6.78	
72	M	250	239.0	−11.0	−4.40	
Group II. 10 days on scurvy diet						
73	M	198	197.5	−0.5	−0.25	−6.3
74	M	248	236.4	−11.6	−4.68	
75	M	252	237.0	−15.0	−5.95	
Group III. 15 days on scurvy diet						
77	M	235	221.0	−14.0	−5.96	−4.5
78	M	250	234.0	−16.0	−6.40	
76	M	255	252.0	−3.0	−1.18	
Group IV. 19 days on scurvy diet Definite symptoms of beginning scurvy						
86	M	205	176.9	−28.1	−13.7	−16.3
82	F	225	182.8	−42.2	−18.8	
Group V. 21–54 days on scurvy diet Death from severe scurvy						
84	M	176	117.4	−58.6	−33.3	−37.0
85	M	228	118.0	−110.0	−48.3	
143	M	283	144.5	−138.5	−48.9	
122	F	295	167.8	−127.2	−43.1	
121	M	314	205.0	−109.0	−34.7	
130	F	386	267.0	−119.0	−30.8	
148	M	377	288.0	−89.0	−23.6	
123	F	438	291.0	−147.0	−33.6	
Group VI. 19–22 days on scorbutic diet. Then on antiscorbutic diet until apparently cured						
80	F	214*	158.9	−55.1	−25.7	+68.2
81	M	217*	253.5	+36.5	+16.8	
43	M		517.7			
289	M	314*	555.8	+241.8	+77.0	
292	M	325*	621.3	+296.3	+99.2	
290	M	304*	684.5	+380.5	+125.0	
294	M	328*	712.5	+384.5	+117.0	

* Maximum during period of scurvy diet.

to 54 days with a very severe type of scurvy. There were swellings of the wrists, knees and costochrondral junctions, intramuscular hemorrhages around the shoulders, knees and ribs, and "white lines" at the knees and costochondral junctions. The teeth were loose, the suprarenal glands very large and reddish yellow, the lungs and intestines congested.

Group VI. Seven animals were fed scorbutic diet 19 to 22 days and were then cured on orange or rhubarb juice with green feed given 18 days to 4 months. Autopsy showed permanent deformity of the osseous structures in only a few cases. The organs otherwise appeared normal.

With only one exception, the animals which did not die from scurvy were chloroformed. The autopsy was performed immediately after death. The head was sectioned from the body at the occipito-vertebral junction and the body suspended to drain the blood, while the brain, hypophysis and eyeballs were dissected clean from the skull. The thyroid gland was then carefully dissected out. Then the thorax was opened, the thymus (not included in the present study) excised, and the heart detached, opened and emptied of blood. The lungs, liver, spleen, pancreas, stomach, intestines, kidneys, suprarenals, testes or ovaries, and bladder were removed with as little adherent connective tissue as possible. The spinal cord was obtained by removing the roof of the bony canal, opening the dura and severing the nerve roots as close to the cord as possible. The organs were all kept in a closed container on moist filter paper and weighed on a balance accurate to the tenth of a milligram. The weights of the organs in the test animals are compared with the weights of normal organs (with corresponding body weight) as taken from the normal curves established by Bessesen and Carlson ('22) which show the normal growth of various organs in the guinea pig. In the following three tables are given the individual data for the body weight (table 1) and the suprarenals (table 2), and the group averages for the other organs (table 3). While the number of test animals used is relatively small, it appears sufficient to justify at least tentative conclusions as to the effects upon the weights of the various organs.

On comparing the initial with the final body weights (table 1) it is evident that there has been a slight loss, averaging only 5 or 6 per cent, in the first three test groups (up to 15 days). The loss increases to 16.3 per cent in the slightly scorbutic group (at 19 days) and to 37.0 per cent in those dying from scurvy. In the cured group VI, the body weight averages 68.2 per cent above the maximum during the period of scorbutic diet.

Since a scorbutic diet results in malnutrition, usually involving a marked loss in body weight, the question has frequently been raised as to whether the effects of scurvy may not be due, at least in part, to general inanition (ordinary starvation). Evidence upon this question is available by comparing the changes in weight produced by the two conditions in the various organs. The results of Lazareff ('95), who studied the weight changes in various organs (brain, spinal cord, heart, lungs, liver, spleen, kidneys, pancreas, stomach, intestines, bladder and skin) of guinea pigs starved with loss in body weight of 10 per cent to 36 per cent, are especially valuable for comparison in this connection.

For the organ weights of the present series (table 2 and 3), it must be remembered that the percentage differences do not represent the actual changes in weight, but merely the relation of the final organ weight to the normal for the corresponding final body weight.

Suprarenal glands (table 2). In groups I, II and III, the suprarenals usually appear slightly above normal in weight, but not much more than would result if these organs remained constant while the body weight decreased slightly. Making allowance for individual variations, it would therefore appear that the suprarenals maintain their original weight during the first 15 days on scorbutic diet. In stage IV at 19 days (beginning scurvy) with a loss in body weight of 16.3 per cent, the suprarenal glands average 78.8 per cent above normal. In group V (severe scurvy) with average loss in body weight of 37 per cent the weight of the suprarenals averages 270 per cent above the normal organ weight for corresponding (final) body weight. This would indicate that the enlargement of the suprarenals begins at about the same time that the symptoms of scurvy appear, but it becomes much greater in the later stages (in accordance with the observations of McCarrison). As may be noted in group VI, in some cases the hypertrophy of the suprarenals persists for some time after the scurvy is apparently cured.

Jackson ('15, '15a) found a slight increase in the weight of the suprarenals in underfed young rats and little or no loss in adult rats during starvation. Enlargement of the suprarenals was noted by Rondoni and Montagnani ('15) and Rondoni ('19) in guinea pigs starved or on diets of oats or maize.

McCarrison ('21) summarizing his earlier investigations (Indian Journ. Med. Research, 1919) found that the suprarenal glands enlarge in consequence of all classes of deficient dietaries, including total inanition as well as various vitamin deficiencies. In five guinea pigs fed on scorbutic diet, the average body weight decreased from 528 to

364 grams, a loss of about 31 per cent. The average weight of the suprarenals at autopsy was 0.955 gram, or practically double that in four normal controls (0.467 gram) of 627 grams average body weight.

TABLE 2

Suprarenal glands

STAGE OF EXPERIMENT	BODY WEIGHT IN GRAMS	WEIGHT OF SUPRARENALS IN GRAMS	NORMAL FOR CORRESPONDING BODY WEIGHT IN GRAMS	DIFFERENCE IN GRAMS	DIFFERENCE PER CENT	AVERAGE PER CENT DIFFERENCE FOR GROUP
5 days diet	180.9	0.1032	0.086	+0.0172	+20.0	+8.1
	227.5	0.0956	0.110	−0.0144	−13.1	
	239.0	0.1338	0.114	+0.0198	+17.4	
10 days diet	197.5	0.0858	0.095	−0.0092	−9.7	−7.4
	236.4	0.1317	0.113	+0.0187	+16.6	
	237.0	0.0916	0.113	−0.0214	−19.0	
15 days diet	221.0	0.1182	0.107	+0.0112	+10.5	+9.0
	234.0	0.1148	0.112	+0.0028	+2.5	
	252.0	0.1008	0.117	−0.0162	+13.9	
Beginning scurvy (19 days)	176.0	0.1502	0.083	+0.0672	+81.0	+78.8
	182.8	0.1553	0.088	+0.0673	+76.5	
Scurvy with death (21 to 54 days)	117.4	0.1798	0.050	+0.1299	+260.0	+270.1
	118.0	0.2733	0.052	+0.2213	+426.0	
	144.5	0.3049	0.065	+0.2399	+369.0	
	167.8	0.4049	0.078	+0.3269	+419.0	
	205.0	0.2871	0.099	+0.1881	+190.0	
	267.0	0.4230	0.122	+0.3010	+247.0	
	288.0	0.2890	0.130	+0.1590	+123.0	
	291.0	0.2977	0.131	+0.1667	+127.0	
Scurvy with cure	158.9	0.1480	0.074	+0.0740	+100.0	+29.3
	253.5	0.1916	0.118	+0.0736	+62.4	
	517.7	0.4645	0.227	+0.2375	+105.0	
	555.8	0.2205	0.258	−0.0375	−14.6	
	621.3	0.2750	0.327	−0.0520	−15.9	
	684.5	0.3390	0.403	−0.0640	−15.9	
	712.5	0.3695	0.438	−0.0685	−15.6	

Per kilo of final body weight, the suprarenals increased from 0.745 gram in the controls to 2.633 grams in the test animals. This is a difference of about 253 per cent, which corresponds closely with my

result of 270 per cent in severe scurvy among younger (smaller) guinea pigs with slightly greater average loss in body weight (37 per cent). Similarly marked enlargements of the suprarenals were found by McCarrison in pigeons during total inanition or polyneuritis (autoclaved rice diet), and in monkeys on variously deficient diets. The changes in all cases are ascribed to inanition, the vitamins being "but links in the chains of materials requisite for perfect nutrition."

Enlargement of the suprarenal glands in scorbutic guinea pigs has also been observed by Bassett-Smith ('20). La Mer and Campbell ('20) found an increase in weight of the suprarenals proportional to the time during which the scrobutic diet is fed, but no increase during starvation. Vincent and Hollenberg ('20), however, found during inanition the weight of the suprarenals doubled in pigeons and dogs, and an even greater hypertrophy in rats.

Brain (table 3). In the first three groups, the average brain weight of the scorbutic guinea pigs appears nearly stationary, making allowance for individual variations. In the fourth group (at 19 days), it averages 12.4 per cent above normal, which is slightly less than would be expected if it had remained stationary in weight while the body weight decreased 16.3 per cent. In the fifth group (dead from scurvy) the brain apparently averages only 10.4 per cent above normal, although the loss in body weight averages 37 per cent.

This would indicate an actual marked loss in the absolute weight of the brain, though proportionately less than in the body as a whole. This loss in brain weight during scurvy is apparently greater than in starvation as reported by Lazareff ('95). He found an apparent loss of only about 6 per cent in the brain weight of guinea pigs starved with average loss of about 36 per cent in body weight. Similarly in the rat and other animals there is little or no apparent loss in brain weight during starvation (Jackson, '15). Bessesen and Carlson find an unusual amount of variability in the normal brain weight of the guinea pig, however, which may possibly explain the marked apparent loss in the scorbutic series as due to individual variations.

Spinal cord (table 3). The weight of the spinal cord appears above normal, varying from 28 to 69.8 per cent in the various groups. In part, this is most probably because the technique involved in the dissection included more of the tissues (nerve roots, meninges, etc.) than did that of Bessesen and Carlson, who established the norms with which my scorbutic animals were compared. If we admit this explanation and deduct a corresponding amount (about 30 per cent) for each of

the various groups, we find that the weight of the spinal cord has apparently remained at approximately its original absolute weight. An increase in relative weight of the spinal cord would still be expected in the last two groups, but that in the fourth group is still too high for the corresponding loss in body weight. As there are only two animals in this group, allowance must be made for possible individual variations.

TABLE 3

Average percentage differences of organ weights compared with the normal. Final body weights compared with the initial weight; organs compared with normal for corresponding final body weight

PARTS	5 DAY TEST	10 DAY TEST	15 DAY TEST	BEGINNING SCURVY (19 DAYS)	DEATH FROM SCURVY (21-54 DAYS)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Body weight	−5.8	−3.6	−4.5	−16.3	−37.0
Suprarenals	+8.1	−7.4	+9.0	+78.8	+270.1
Brain	+2.8	+0.3	−0.4	+12.4	+10.4
Spinal cord	+30.6	+40.6	+28.0	+62.0	+69.8
Eyeballs	+8.9	+6.8	+7.0	+20.8	+27.5
Thyroid	+14.3	+17.5	+6.4	+68.8	+21.2
Heart	+2.5	+2.4	−0.6	+11.1	+8.6
Lungs	−9.8	−5.8	+12.7	+4.8	+60.3
Liver	−25.0	−24.7	−18.0	+9.7	−1.0
Spleen	−20.8	−38.6	−18.2	+56.3	+35.0
Stomach-intestines (filled)	−7.0	+41.3	+23.2	−1.3	+7.1
Stomach-intestines (empty)	−25.2	−19.9	−8.4	+3.9	+36.3
Stomach	−3.5	−15.3	−10.7	+20.7	+7.9
Pancreas	+14.3	−13.7	−1.0	+29.6	−1.2
Kidneys	+9.9	+22.0	+7.0	+38.0	+57.7
Testes	+41.7	+31.1	+35.6	+76.4	+4.8
Epididymides	+47.2	+42.2	+45.4	+71.4	+75.2
Ovaries				−7.6	−19.8
Bladder	+37.3	+12.6	+9.7	+29.7	+44.7
Hypophysis	+8.0	+9.2	+12.9	+12.8	+50.5
Integument	−11.3	−19.4	−16.8	−13.0	−1.8

No data appear in the literature as to the weight of the spinal cord during scurvy. Lazareff ('95) found in the guinea pig during starvation a very slight loss in the weight of the spinal cord, similar to that of the brain.

Eyeballs (table 3). The average weight of the eyeballs appears above normal in all groups. A comparison of the corresponding body weights indicates that the eyeballs have maintained very nearly their original

absolute weight, the apparent condition above normal being accounted for by the loss in body weight. No data have been recorded in the literature concerning the weight of the eyeballs of the guinea pig, either during scurvy or starvation. In other species, however, the eyeballs, like the nervous system, suffer little or no loss in weight during starvation (Jackson '15).

Thyroid gland (table 3). The thyroid gland appears above normal weight in all groups, but on account of the normal variability and the difficulty in the technique of dissection it is doubtful whether the apparent increase is significant. The results, therefore, do not confirm McCarrison's conclusion that the thyroid gland in guinea pigs on scorbutic diet increases to double or triple the original normal weight. In other types of deficiency (including total inanition) McCarrison found a tendency to atrophy in the thyroid gland of the monkey and pigeon. Jackson ('15) found a greater loss during chronic inanition than during acute inanition in the adult albino rat.

Heart (table 3). The heart maintains nearly normal weight in all groups, indicating a loss in weight almost proportional to the loss in body weight. McCarrison observed a relative decrease in the weight of the heart in scorbutic guinea pigs. On the other hand, La Mer and Campbell ('20) found some evidence indicating a hypertrophy of the heart in guinea pigs on scorbutic diet, which would accord with the findings in human scurvy, according to Hess ('20). Lazareff ('95) found that during total inanition in the guinea pig the decrease in the weight of the heart is nearly proportional to that of the whole body. The same holds true for the rat, with water, according to Jackson ('15).

Lungs (table 3). The lungs in my scorbutic animals show an average weight not far from normal, excepting group V (severely scorbutic) with weight 60.3 per cent above normal. McCarrison found a slight increase in weight of the lung in scorbutic guinea pigs. According to Hess ('20), pneumonia is a very frequent terminal infection in human scurvy, and this might explain the increased weight in my group of guinea pigs dead from scurvy. Lazareff ('95) observed a slight loss in absolute weight (with corresponding increase in relative weight) in the lungs of guinea pigs subjected to various degrees of starvation.

Liver (table 3). The liver in the guinea pigs on scorbutic diet shows a peculiar change in weight. In the earlier stages it averages about 25 per cent below normal, but recovers nearly normal weight after symptoms of scurvy appear. This corresponds in general with the observations of Lazareff ('95) on guinea pigs subjected to various degrees of

starvation, the liver at first suffering a loss in weight relatively greater than that of the whole body, but in later stages returning to its normal relative weight. La Mer and Campbell ('20) find the liver weight unaffected by scorbutic diet. McCarrison, however, finds a marked decrease in both absolute and relative weight of the liver in guinea pigs on scorbutic diet.

Spleen (table 3). The spleen is notably variable in weight, even during health. In the first three groups on scorbutic diet there is a distinctly subnormal weight of spleen, which becomes apparently markedly above normal after scorbutic symptoms appear. A part of this increased weight is doubtless due to the marked hyperemia of the spleen which is evident at autopsy. According to Hess ('20), in human scurvy the spleen is usually enlarged and congested. McCarrison, however, found a marked loss of both absolute and relative weight in the spleen of scorbutic guinea pigs, as well as in various forms of inanition in the monkey and pigeon. In the guinea pig during starvation Lazareff ('95) found that the loss in weight of the spleen is roughly proportional to that of the body as a whole. Jackson ('15) cites evidence showing that during inanition in the rat and other species the spleen in general loses heavily in weight.

Stomach and intestines (table 3). When weighed with contents, these organs show an increased weight shortly after the animal has been placed on scorbutic diet (at the 10 day period), which decreases to normal before symptoms of scurvy appear. On the other hand, the empty gastro-intestinal tract appears about 25 per cent subnormal in weight in the first group, increasing progressively so as to reach approximately normal weight in the fourth group (beginning scurvy) and becoming 36.3 per cent above normal in the fifth (severely scorbutic) group. The (empty) stomach alone shows a less marked initial decrease and also a slighter increase later. The increased weight of the alimentary canal in the later stages is doubtless due in part to the characteristic congestion observed at autopsy, and likewise noted by Hess ('20) and McCarrison ('21). In guinea pigs subjected to various degrees of starvation, Lazareff ('95) found that the stomach loses but little in absolute weight. The loss is greater in the weight of the intestines, but proportionately less than in the body as a whole. Jackson ('15) found during inanition in the adult albino rat a decrease in weight of stomach and intestines relatively greater than that in the body as a whole.

Pancreas (table 3). The data for the various groups appear variable

and inconclusive as to any marked change in the weight of the pancreas of scorbutic guinea pigs. Lazareff ('95) found the decrease during starvation proportional in general to that of the body. McCarrison finds a marked atrophy of the pancreas in pigeons and monkeys fed variously deficient diets.

Kidneys (table 3). All groups of my series show the kidney weight above normal. The increase is slight in the earlier stages, but very marked after scorbutic symptoms appear, and extreme (+ 57.7 per cent) in the severely scorbutic group. La Mer and Campbell ('20) likewise found the kidney weight increased in scurvy, which would agree with the condition of congestion found in the human kidney during scurvy, according to Hess ('20). McCarrison, on the other hand, observed a slight decrease in both relative and absolute weight of the kidneys in guinea pigs on scorbutic diet, as well as in other species on deficient diets. Jackson ('15) found during inanition in the adult albino rat a decrease in kidney weight proportional to the body weight.

Ovaries (table 3). The observations indicate an atrophy of the ovaries during scurvy, but the data are too few to warrant a definite conclusion.

Testes (table 3). The testes appear markedly above normal in all excepting the fifth (markedly scorbutic) group. This condition is surprising and difficult to interpret. In the fifth group it appears nearly normal, as likewise in the cured group (not included in the table). Jackson ('15) noted during inanition in rats a decrease in the testis weight proportional to body weight. McCarrison ('21) likewise observed atrophy of the testis in deficiently fed pigeons and monkeys.

Epididymides (table 3). The epididymides in the various test groups show the same enlargement as the testes, excepting the severe scurvy group, which for the epididymides shows an average weight of 75.2 per cent above the normal organ weight for the corresponding body weight. No data upon the weight of the epididymis in scurvy are available in the literature for comparison.

Hypophysis (table 3). The hypophysis weight averages above normal in all the groups, but none except the last group appears significant. In severe scurvy, however, it averages 50.5 per cent above normal for corresponding body weight. Since there is a loss of 37 per cent in body weight in this group, it is evident that in absolute weight the hypophysis has probably remained nearly unchanged in this as in the other groups. During inanition in the rat, Jackson ('15) found in starved rats a decrease in the weight of the hypophysis nearly proportional to that of the body. McCarrison, however, found but slight atrophy, and in some cases a hypertrophy on deficient diets.

Bladder (table 3). The urinary bladder appears above normal in all the groups. The high figure (+37.3 per cent) for the first group is probably due to accidental variation and therefore without significance. Otherwise the results do not differ greatly from what might be expected if the bladder were to remain nearly constant in absolute weight, while the body weight decreased in the various groups. McCarrison gives no weights for the bladder in scorbutic guinea pigs, but observed a marked congestion. This might tend to counteract a loss in weight from inanition-atrophy. The data of Lazareff ('95) indicate during total inanition in the guinea pig a loss in the weight of the bladder, but proportionately somewhat less than in the body as a whole.

Integument (table 3). In all the groups of my test animals, the skin is below normal, especially in the first four groups. This would indicate that the loss in weight of the integument is somewhat greater proportionately than in the body as a whole, although for the last (severely scorbutic) group the difference is insignificant. Allowance must be made for unavoidable differences in the technique of removal of the skin, as well as for individual variations. The data of Lazareff ('95) indicate that the loss of weight in the skin of the guinea pig during starvation is proportionately less than in the body as a whole. In the adult albino rat, Jackson ('15) found the loss nearly proportional to that of the body, the relative weight therefore remaining unchanged.

DISCUSSION AND SUMMARY OF RESULTS

The effects of a scorbutic diet upon the weights of the various organs of the guinea pig evidently vary greatly in the different viscera and also according to the length of the experiment. In most cases, during the first 15 days, the changes in weight are comparatively slight and probably of no significance. The spinal cord, testes, epididymides and urinary bladder appear markedly above normal in weight during this early period, however, while the liver, spleen, stomach-intestines and integument are definitely subnormal.

In the later stages, after the symptoms of scurvy are apparent, the changes in organ weight (as well as in body weight) are more evident. In comparison with the normal for corresponding (final) body weight, the ovaries alone appear definitely subnormal. The pancreas (late stage), heart, liver, testes (late stage) and integument appear nearly normal, indicating a loss in weight roughly proportional to that of the entire body. The brain, eye-balls, thyroid gland, spleen and intestines appear somewhat above normal, indicating some loss in absolute

weight, but in most cases proportionately less than that of the entire body. The spinal cord, lungs (late stage) kidneys, epididymides, hypophysis (late stage) and urinary bladder are markedly above normal, indicating little or no loss in absolute weight, in spite of the great loss in body weight. The suprarenal glands have undergone a tremendous increase in absolute as well as in relative weight, so as to average 78.8 per cent above normal for corresponding body weight in the beginning scurvy group (with loss of 16.3 per cent in body weight) and 270.1 per cent above normal in those dead from scurvy (with 37 per cent loss in body weight.) The organs markedly above normal weight are usually congested, which may at least partly account for their increased weight. These results in general confirm and extend (to other organs) the observations of McCarrison and others, the differences being explainable partly on account of accidental variations, and partly as due to differences in technique, age of animals used, etc. More precision has been possible in the present work on account of having the norms of Bessesen and Carlson available for comparison.

The results also show a general agreement with those produced by starvation, somewhat tending to confirm McCarrison's view that the changes during scurvy (and other dietary deficiencies) are explainable as due to general inanition. The results in the two conditions are by no means identical, however, and marked differences in some of the organs (brain, lungs, spleen, kidneys, testes, epididymides and bladder) indicate that the scorbutic condition involves factors producing changes in weight different from those in ordinary inanition.

BIBLIOGRAPHY

Bassett-Smith. '20. Proc. Roy. Soc. Med. London, July, no. 9 (War Section), xiii, 51.

Bessesen, A. N., Jr. and H. A. Carlson. '22. Postnatal growth in weight of the body and of the various organs in the guinea pig (unpublished).

Hess, A. F. '20. Scurvy, past and present. Philadelphia and London.

Jackson, C. M. '15. Amer. Journ. Anat., xviii, 75.

'15 a. Journ. Exper. Zoöl., xix, 99.

La Mer, V. K. and H. L. Campbell. '20. Proc. Soc. Exper. Biol. and Med., xviii, 32.

Lazareff (Lasarew), N. '95. Dissert, Warsaw.

McCarrison, R. '21. Studies in deficiency disease. London. Also '19, Indian Journ. Med. Research, vi, 275, 550; vii, 188, 308.

Rondoni, P. '19. Brit. Med. Journ., May 3, 542.

Rondoni, P. and M. Montagnani. '15. Lo sper., lxix, 659.

Vincent, S. and M. S. Hollenberg. '20. Endocrinol., iv, 408.

THE ACETONITRIL TEST FOR THYROID AND OF SOME ALTERATIONS OF METABOLISM*

REID HUNT

From the Laboratory of Pharmacology, Harvard Medical School, Boston[1]

Received for publication October 18, 1922

I. CAUSE OF THE ACETONITRIL REACTION AND SOME OF THE FACTORS MODIFYING IT. Attention has been called in a number of earlier papers (Hunt 1907a, 1910, 1911) to the extremely great variations in the toxicity of acetonitril (methyl cyanide) caused by variations in diet, the administration of thyroid and by various other agencies affecting metabolism. Especial attention was devoted to the effects of thyroid and upon these was based a delicate test for the presence of this substance and for the comparative physiological activity of different preparations of the gland and of substances derived from it (Hunt, 1905, 1907c, Hunt and Seidell, 1908a, 1908b, 1910). It is proposed to review, in the present communication, certain features of this test as applied to the thyroid and to describe some recent applications of it.

The test for thyroid is based upon the greatly *increased* resistance of mice to acetonitril, injected subcutaneously, when thyroid is administered to them, usually per os. The administration of thyroid to other animals tested causes a *lowered* resistance to the poison whereas

*CONTENTS. 1. Cause of the Acetonitril Reaction and Some Factors Modifying It. Introduction. *Toxicity of Acetonitril—Effects of Undernutrition—Alcohol—Age, sex, light and season—Diet;—vitamins—bread.*

2. The Acetonitril Test for Thyroid—Method, Choice of Animals, Diet.

a. Review of previous work—The reaction as a test for the presence of thyroid—The relation between the iodin content and the physiological activity of thyroid; pharmacopoeial standards. Iodization of thyroid *in vivo*. "Inactive iodin" in thyroid. "Iodin-free" thyroid. Fetal thyroid. Thyroid of exophthalmic goiter.

b. Thyroxin; activity of as compared with thyroid.

3. The Acetonitril Reaction as a Test for Thyroid Secretion. Graves' Disease. Chronic Nephritis. Blood of Thyroidectomized Animals.

[1] These experiments are a continuation of an investigation begun in coöperation with Doctor Renshaw with the aid of a grant from the Committee of the Permanent Charity Fund, Incorporated. The experiments are being continued.

other agencies affecting metabolism affect mice and other animals in the same way. It is this anomalous reaction of mice to thyroid that gives the test its value; for example, if an unknown substance increases the resistance of mice but lowers that of rats to the nitril the probability is that the substance is thyroid or a derivative of thyroid. Similarly, if there are indications that some agency increases metabolism but lowers the resistance of mice to acetonitril the probabilities are very great that the agency does not affect metabolism through an effect upon the thyroid gland. It is obvious that such a test is very desirable since at the present time there is a tendency to attribute many changes in metabolism to hyper- or hypoactivity of the thyroid gland.

Thus while the primary purpose of this paper is to discuss the acetonitril test for thyroid it is obviously necessary to consider the action of the nitril from a broader standpoint; in fact the effect of thyroid is but one phase of the relation of acetonitril to metabolism.

The *toxicity* of *acetonitril*[2] depends upon two actions. As regards its immediate effects acetonitril belongs to the alcohol group of narcotics but its more characteristic actions are due to its decomposition products; the situation is analogous to that of methyl alcohol, the immediate effects of which are similar to those of other members of the alcohol group but the later (and more deleterious) effects of which are probaby due, directly or indirectly, to its oxidation products (formic acid and perhaps formaldehyde) (Hunt, 1902). In the case of acetonitril the most poisonous of its decomposition products in the body is hydrocyanic acid; part of this is neutralized by sulphur and excreted as thiocyanate. The methyl group of acetonitril appears in the urine as formate. The formation of these products is apparently due to the hydrolysis of the acetonitril molecule but there are indications that simultaneous processes of oxidation are also involved (Hunt, 1904, 1907a).

It was in connection with a study of the toxic action of acetonitril that I discovered the test for thyroid (1905). I thought that if certain processes of oxidation in the body could be increased the toxicity of the nitril should be increased as a result of the freeing of a larger amount of hydrocyanic acid. Accordingly I administered thyroid to rats and guinea pigs; the toxicity of the nitril was greatly increased. But mice, both white and gray, reacted in the opposite way: they be-

[2] For a fuller discussion of this subject and also of the relation of other nitrils to the thyroid see Hunt (1904); also in Heffter's Handbuch der exper. Pharmakol. I (in press).

came very resistant to the poison. I have been unable to find a very probable explanation of this species difference.

Under other conditions which had been suggested as modifying oxidation processes in the body the various animals reacted alike. Thus the suggestion had been made that in conditions of *undernutrition* the body "could use less material and work more economically." Hence I thought that it would be of interest to test the acetonitril resistance of animals on a restricted diet, the hypothesis being that if processes of oxidation were lowered in this condition less hydrocyanic acid would be formed and the resistance of the animals increased. As a matter of fact, the resistance of both mice and guinea pigs, on a restricted diet, was much increased. There was also a diminution in the amount of thiocyanate excreted; this indicated that less hydrocyanic acid had been formed from the nitril, for inanition had no effect upon the amount of thiocyanate excreted when this had been administered. Inanition also did not increase the resistance of animals to hydrocyanic acid. I concluded accordingly that the increased resistance to acetonitril in conditions of undernutrition was probably "dependent upon a lessened breaking up of the acetonitril molecule and this again upon a diminution in the intensity of those processes of metabolism by which the acetonitril molecule is decomposed."[3]

Miura (1922 a) has recently confirmed my experiments in which a limited oats diet was used but obtained negative results when a "standard diet" of purified foodstuffs was fed. Doctor Renshaw and I, however, in 1919, in some unpublished experiments, found an increased resistance of mice on a limited diet of purified food stuffs (casein 18, starch 45, yeast 5, lard 18, butter 9, salts 5): the fatal dose of the nitril for mice on an unlimited amount of this diet was 0.15 mgm. per gram or less (all mice dying); that for those on limited amounts of the diet was 0.3 mgm.

Reasoning somewhat similar to the above led to experiments in which acetonitril was administered to animals which had received, for some time, small doses of alcohol (1907 a). Alcohol was supposed at that time to "lower processes of oxidation" in the body but it seemed to me that perhaps the tolerance so readily established to alcohol may be due to the body having acquired the ability to

[3] It has been suggested in connection with the recently demonstrated lowering of the basal metabolism of man in conditions of undernutrition that the thyroid gland becomes less active; this explanation would not hold for the mouse, however, for in this animal a diminution of the activity of the thyroid leads to a lowered instead of an increased resistance to acetonitril. Similarly the increased metabolism in dogs resulting from "overnutrition" has been attributed to overactivity of the thyroid. But, as will be shown below, those diets and some other agencies and conditions which may be assumed to stimulate metabolism lower the resistance of mice to the nitril; if the thyroid of the mouse is overactive the resistance to the nitril is increased. These results are in harmony with the point of view especially emphasized in this country by Benedict that basal metabolism is ordinarily governed by the mass of active protoplasm rather than with the theories which attribute so many changes in metabolism to hypothetical alterations in the activity of the thyroid.

oxidize it more rapidly or more completely; although this was a rather obvious hypothesis I could not find that it had been suggested or that any work of any kind had been done on the question of tolerance to alcohol. If the tolerance to alcohol is due to an increased ability of the body to oxidize the drug then it seemed possible that an animal tolerant to alcohol might also oxidize the methyl group of acetonitril more rapidly and free more hydrocyanic acid and so be less resistant to the poison. As a matter of fact, it was found that animals which had received for some time very small amounts of alcohol were less resistant to the nitril. That this was probably due to a greater formation of hydrocyanic acid in the body was shown by the fact that there was an increased excretion of thiocyanate in these animals. Such animals were not less resistant to hydrocyanic acid itself. Thus the results were just the reverse of those found in animals on a restricted diet. These experiments also supported the hypothesis that the tolerance to alcohol is accompanied by an increased ability of the body to oxidize it—a hypothesis later confirmed by the experiments of Pringsheim (1908).

Other factors which at that time, or subsequently, have been believed, or shown, to be accompanied by an increased basal metabolism led to a lowered resistance to the nitril: thus young animals were less resistant than adults; males were less resistant than females; mice kept in a cage with a wheel upon which they exercised a great deal (by which the total muscular mass was doubtlessly increased) were somewhat less resistant than mice kept on the same diet in jars where there was less opportunity for exercise; in general the more rapid the growth of an animal, the lower was the resistance.

Sundstroem (1922), in experiments apparently performed with unusual care, has recently found that *light* accelerates the growth of white mice and also diminishes their resistance to the nitril. As will be mentioned below, I had found that cod liver oil has similar effects. These results are of interest in connection with the recent work on the relation of light and cod liver oil to rickets.

Season also has a marked effect upon the nitril resistance of mice: the latter are much more resistant in the winter and spring than in the summer and autumn months. Some data were presented in an earlier paper (1910) which suggested that these seasonal variations are connected with varying degrees of thyroid activity; it is well known from the observations of Koch (1907) and others and especially from the work of Seidell (1911) and of Seidell and Fenger (1913, 1914) that there are marked seasonal variations in the iodin content of the thyroids of various animals (beef, hog and sheep but not, apparently, in those of the dog). Sundstroem makes the interesting and plausible suggestion that seasonal variations in nitril resistance may be due to the influence of light.

The most interesting and, from the standpoint of applying the test, the most important factor, aside from thyroid itself, affecting the resistance of mice to acetonitril, is the *diet*. As I showed many years ago (1905, 1910, 1911) the resistance of mice to this poison may be made to vary at will by feeding different diets; animals have been made forty times as resistant as others by this means. I early recognized that these effects, as well as the effects of diets upon the growth and repro-

duction of mice, could not be explained on the basis of differences in the amount of "protein, fat, carbohydrates and salts"—the dietary factors receiving most attention at that time—and was led to remark (in 1910) "although there is a vast accumulation of the most accurate knowledge of foods from the dynamic and economic points of view, little is known of the specific action of the various foods" and that "it can scarcely be said that even the theoretical basis for the choice of the various foods in health or disease has been laid"[4] and that it seemed probable that by the use of the nitril reaction "changes in metabolism which are not recognized by methods ordinarily employed, may be detected."

Little progress has been made in explaining the effects of diet upon the acetonitril reaction. I was able to show that in a few cases there was probably some relation between some substance in the food and the activity of the thyroid gland; in other cases sulphur compounds seemed to be involved; I also called especial attention to the fact that those diets which caused most rapid growth and reproduction usually led to a low degree of resistance but that the reverse did not always hold: a low degree of resistance and slow rate of growth resulted from diets containing much of certain fats.

Miura (1922a) has recently confirmed, by carefully controlled diets, my early inferences that variations in the relation of protein, carbohydrate and salts can not explain the variations in resistance. He also states that the vitamins (A and B) are not involved. Doctor Renshaw and I had done considerable work on this latter problem but had not reached a definite conclusion. We had obtained marked differences in the resistance of mice to acetonitril when the only difference in the diets was the presence or absence of foodstuffs supposed to act, under the circumstances, only in virtue of their "vitamin" content but we were unable to determine how much of this effect was to be attributed directly to the "vitamins" and how much to the increased rate of growth which follows the use of these diets. The results of such an experiment are shown in table 1.

The tests were made when the mice had been on the special diets for only a week and before the effects of vitamin deficiency had become

[4] The experiments upon which these conclusions were based were performed before the terms "vitamins," "food accessories," etc., had been coined. In fact, this work antedated all of the "newer" work on nutrition with the exception of Hopkins' preliminary report (1906); many of my experiments, as the published protocols show, were, however, performed in 1905.

marked; it was thought that in this way any specific effect of the vitamin might be more distinct. (If the vitamin-free diet is continued there is, with the rapid loss of weight, a lowering of the resistance to the nitril.)

TABLE 1

	CHANGE IN WEIGHT	FATAL DOSE OF ACETONITRIL MGM. PER GM. MOUSE‡
	per cent	
Vitamin free diet*	−3.0	0.42
Vitamin free diet plus 5 per cent yeast (replacing equal amount of starch)	+19.8§	0.15 or less†
Vitamin free diet plus 9 per cent butter fat (replacing equal amount of lard)	+7.4	0.15 or less†
Complete diet	+20.6	0.15 or less†

* Purified casein 18, starch 50, lard 27, salts 5. (Osborne and Mendel, Journ. Biol. Chem., 1919, xxxvii, 572.)

† All mice died from this and larger doses; lower doses were not given.

‡ The nitril was dissolved in water and injected subcutaneously.

§ I obtained similar results from the feeding of cracker dust to which yeast had been added: marked acceleration of growth and a lowered resistance to the nitril; the experiments were performed in 1907 and published in 1910. So far as I am aware, this was the first time that attention was directed to the "growth-promoting" properties of yeast.

In an effort to determine whether the effect of yeast noted above is to be ascribed to its "vitamin" content I have performed a number of experiments with a commercial yeast vitamin preparation[5] made accord-

[5] The commercial preparation was prepared by the Harris Laboratories and obtained upon the open market; it was used both in the tablet form and as a dried powder. Since the quality of the Harris preparation has been questioned by McCollum and Simmonds (1922) it was necessary to consider their criticisms in order to determine whether any importance was to be attached to my experiments with it. McCollum and Simmonds stated that "the tablets (of yeast vitamin-Harris) contained at most but a trace of the vitamin for which they are recommended." This statement is based upon experiments in which the dosage of the vitamin for rats was based upon the recommended dosage for man; whether this refers to single or to daily doses is not stated. McCollum and Simmonds found that three times the relative dose for man had no effect upon the growth of the rat but that twenty times caused a "fair response" as to growth. The dose of the Harris preparation recommended for man is 400 mgm., at a single dose, or 1.2 gram per day. Accepting McCollum and Simmonds' assumption that this is for a man of 70 kgm. this would correspond to 5.7 mgm. per kilo for a

ing to the method of Osborne and Wakeman (1919) and also with a preparation kindly sent me by Dr. Atherton Seidell which had been prepared by his fuller's earth method (Seidell, 1922). When these preparations were added to the extent of 1 per cent (smaller amounts were not tested) to a somewhat inadequate diet (special dog bread) there was uniformly a lowering of the resistance, the fatal dose of the nitril for the vitamin-fed mice being but one-half or less that for the controls; the mice were adults and there were slight or no changes in weight.

When "vitamin" and thyroid, or thyroxin, were fed simultaneously there was a distinct antagonism between their effects on the nitril reaction. Thus in a series of experiments upon adult, and apparently old, mice the following results were obtained.

single dose or 17.1 mgm. per kilo per day. Osborne and Wakeman seem to have used from about 100 to 200 mgm. of their preparation per kilo rat or from about 17 to 34 times the relative single doses for man. In any case the larger doses of the Harris preparation used by McCollum and Simmonds seem to have been of about the same order as those used by Osborne and Wakeman and both groups of workers obtained positive results. In other words the activity of the Harris preparation (which I used) seemed to be of about the order as that of the preparation of Osborne and Wakeman and the latter found it to be about 16 times as active as yeast.

It is not clear why McCollum and Simmonds in their tests should have based the dosage of the vitamin for rats upon the recommended doses for man since it is so well known that the requirement per kilo of food stuffs for small animals, like rats and mice, is many times greater than for man. Thus Renshaw's (1921) mice consumed per day per kilo about 27 grams protein, 41 grams fat and 77 grams carbohydrate; the Royal Society Food Committee recommended that a man doing moderate work should receive per kilo per day 1 gram protein, 1.28 gram fat, and 7.8 grams carbohydrate, i.e., the mice required for maintenance from 10 to 27 times as much per kilo of the various foodstuffs as does a man doing moderate work; their vitamin requirements may be correspondingly great. (Hess found that it required about one-third as much orange juice per capita to protect guinea pigs against scurvy as it did to protect infants.)

McCollum and Simmonds compared the Harris preparation in the above dosage with wheat germ which constituted 5 per cent of their rats' diet. If they had assumed that the food of man should contain 5 per cent of wheat germ if this is to serve as the source of the water-soluble vitamin, and then based the dosage of this for rats upon what a man would receive in such a diet they would probably have found wheat germ as inefficient as the Harris preparation.

I know nothing as to the value of the Harris preparation in the doses recommended, or in any dose, for man but it does seem from McCollum and Simmonds' own work that it represents what it is claimed to be: a concentrated preparation of the yeast vitamin and so suitable for experimental work.

DIET	FATAL DOSE OF NITRIL: MGM. PER GM. MOUSE
Dog bread	0.67
Dog bread + 0.004 mgm. thyroxin daily	0.81
Dog bread + 40 mgm. vitamin (Harris) daily	0.40
Dog bread + above amounts of vitamin and thyroxin	0.45

More striking results were obtained when mice, similar to the above, were placed upon much larger doses of thyroxin:

DIET	FATAL DOSE OF NITRIL: MGM. PER GM. MOUSE
Dog bread	0.77
Dog bread + thyroxin, 0.016 mgm. daily	2.5
Dog bread + vitamin (Harris) 40 mgm. per day	0.36
Dog bread + above amounts of vitamin and thyroxin	1.6

The resistance of thyroxin-fed mice was lowered to the same extent by a single intravenous injection of 5 mgm. (per mouse) of Seidell's vitamin preparation.[6]

If these results are really due to the "vitamin" contained in the preparations, this method may open up a new way of detecting changes in intermediary metabolism caused by vitamins.[7] The experiments are being continued using purified foodstuffs.[8]

[6] I called attention in earlier papers to the antagonism between milk and thyroid and suggested that possibly the beneficial effects reported from the administration of the milk of thyroidectomized goats to patients with Graves' disease was due to the milk itself rather than to the presence in it of some hypothetical antibody.

This antagonism between thyroid and milk (or vitamin B) may perhaps be of interest in another connection. I have presented arguments in earlier papers for the view that the thyroids of mice, rats and guinea pigs are more active in the winter than in the summer. Hopkins (1920) has reported that minimal amounts of milk were more effective in the late spring or early summer than in the winter as a source of vitamin B for rats; possibly in the winter the activity of some of the vitamin was neutralized by an increased activity of the thyroid.

[7] It would be especially interesting to test the acetonitril resistance of groups of mice all of which were receiving the same amount of food but some of which were making more rapid gains in weight than others as a result of the addition to their diet of vitamins or of an additional amount of vitamins. The experiments of Hopkins (1912) with rats and of Renshaw (1921) with mice indicating

The experiments recorded in table 1 show that when butter fat (as a source of vitamin A) was substituted for a part of the lard of the basal diet there was increased growth and a lowered resistance to the nitril. This result is similar to those I reported several years ago with another fat rich in what is now called "vitamin A": I found that cod liver oil added to certain diets was more effective in lowering the resistance of mice to the nitril and maintaining, or causing increase in, weight than any other of a number of fats tested.[9]

Miura concluded from his experiments that "the susceptibility to poisoning in mice with acetonitril is not easily affected by changes in dietary composition within wide limits of quantity and especially of quality." But Miura states that he worked only with "standardized, synthetic foods the nutritional significance of every factor of which is known,"[10] and which "was adequate for maintenance or slight gain." That is, he did not work with diets which do cause extraordinarily great changes in the resistance of animals to acetonitril and naturally his experiments throw no new light upon this question. The chief interest in the nitril experiments is that they show that there are factors in the diet the significance of which is not known.[11]

that vitamin increases the animal's ability to utilize its food in the production of growth are perhaps the most significant experiments yet published relating to the physiological action of the vitamins.

[8] In testing the efficiency of vitamin preparations I have for the last two or three years been injecting them intravenously into mice; the Seidell preparation is especially well adapted for this method of administration. Not only is there an immediate improvement in the symptoms of mice on a diet deficient in the vitamin but their life is prolonged far beyond that of those on the deficient diet. This method gives results more quickly and with the use of much smaller amounts of material than the usual rat test.

[9] The relation of fats to the nitril resistance is very complex and has not been sufficiently investigated. To date I have found only a lowered resistance to follow the administration of cod liver oil, butter fat and olive oil; the latter has not the growth-promoting properties of the two former. A lowered resistance, with a loss of weight, has followed the administration of relatively large amounts of corn, egg and cottonseed oils and lard; with small amounts of lard and "Crisco" and almond, cottonseed, linseed and cocoanut oils there has been an increased resistance. The various fats and oils differ much in these effects; thus there seem to be differences in the nutritive values of fats and oils and these can not be explained solely on their "vitamin" content (the chief criterion by which this class of foodstuffs seems to be judged at present).

[10] It may be noted in this connection that Miura reported no experiments on the nitril resistance of mice on diets containing less than 21.6 per cent of fat.

[11] Other lines of work are showing that even the newer knowledge of amino acids, vitamins, etc., does not suffice to explain all the effects produced by food-

Many of the experiments with the nitril have been made with one-sided and more or less "inadequate" diets; the diets were selected from the standpoint of their effect upon the nitril reaction and not from that of their ability to promote growth. But mice were kept for four months and were able to rear a considerable number of young upon the diet (oatmeal and liver) which caused the most extreme change in the resistance to the nitril which I have obtained; four months (I do not know how much longer the experiment could have been continued) of a mouse's life would correspond to a considerable period in a man's life. Burget (1917) states that rats will live indefinitely upon such a diet.

But diets which have been pronounced "adequate" (from the standpoint of growth in rats) may also have marked effects upon the nitril reaction. Thus Renshaw and I (in unpublished experiments) found that a diet consisting of rolled oats 60, starch 20.3, salts 4.7, gelatin 10, butter fat 5[12] made mice resistant to the nitril, the fatal dose in one series of experiments being above 0.75 mgm. per gram; other mice of the same age, etc., kept on an "adequate" diet analogous to the one used by Miura died from 0.15 mgm. per gram. Omission of the gelatin for a week lowered the fatal dose to 0.25 mgm. The gelatin is supposed to supplement the inadequate proteins of the oats; careful analysis of the acetonitril reaction in such an experiment would probably throw light on the physiological processes for which the oats proteins are inadequate and how the gelatin supplements them.

I thought it would be of interest to compare the acetonitril resistance of mice reared upon one of the modern "complete, synthetic" diets of purified foodstuffs and those reared upon diets composed of natural foodstuffs. For the latter I selected *1*, a diet (based upon a formula received from Doctor Castle of the Bussey Institution) which we have used with marked success in breeding experiments and consisting of oatmeal, hominy, dried milk, meat scrap and sodium chloride; *2*, a diet consisting of equal parts of egg yolk, whole powdered milk and oatmeal; *3*, a "special dog bread" to which 10 per cent powdered milk had been added; *4*, a "complete" diet of purified foodstuffs.

Breeders kept on diet *2* reared the most young; the fatal dose of the nitril for their young was 0.13 mgm. per gram; groups *1* and *3* also reared many young; the fatal dose of the nitril for those on *1* was 0.59, for

stuffs, but probably nowhere is there a larger mass of unexplained data than in connection with the effects of diet upon the acetonitril reaction.

[12] cf. McCollum, Simmonds and Pitz (1917).

those on *3* was above 0.4. No young of the mice kept on the "complete diet" reached maturity and so their resistance to the nitril could not be determined.[13] Diets which permit of rapid growth and reproduction are certainly of more interest than those which scarcely more than serve for maintenance.

Numerous other illustrations of the "inadequacy" of the present views of diets could be cited and will be published later but one more illustration may be given in reference to an article of food in widespread use: viz., *bread*. I found in 1910 that mice fed upon one brand of "wheat bread" were uniformly about four times as resistant to the nitril as were mice fed upon another brand; the two brands were considered by the dealers to be identical. There were no constant differences in the effects of these two breads upon the growth of the mice,—which would perhaps be the criterion by which their nutritional equivalence, aside from their chemical analyses, would at the present time be judged. These results led me to remark: "It is evident that for careful dietary studies the exact composition of the bread should be known; it also seems desirable for the words 'white wheat bread' to mean something more definite than they do at present.[14] It is not entirely improbable that in certain cases of sickness different kinds of bread would have different effects analogous to those found in experiments on lower animals."

2. THE ACETONITRIL TEST FOR THYROID. The acetonitril test was developed primarily in connection with a study of thyroid as a drug, that is, when the gland was to be administered by mouth. It has served a useful purpose for detecting minute amounts of thyroid substance, for which there are no satisfactory chemical methods; for determining

[13] Some of the young on diet *1* were transferred to the synthetic diet and their nitril resistance tested a month later; the fatal dose was less than 0.3 mgm. per gram, whereas mice which had continued on diet *1* survived 1 mgm. (These experiments were performed in the autumn when the resistance is higher than in the summer.)

[14] This is even more desirable at the present time for bread now is more complicated and variable than ever; many new ingredients are being added with the idea apparently of making it a more nearly "complete food," the completeness being determined by the ability of rats to grow normally upon it. It is a question if it would not be better to have bread of a simpler and more uniform composition with its limitations as a food clearly recognized. For example, iodides seem to be added to some brands of bread; this hardly seems the logical place to seek the iodin which the body needs and, moreover, iodides are also being added to table salt and it has recently been proposed to add them to candy. The excessive intake of iodin has long been recognized as very deleterious to patients with some forms of goiter and there may be an element of danger in its too free use in articles of food. (See for example Hunt (1907 b) and the impressive warning by Bircher (1922).)

the relation between physiological activity and iodin content, upon which was based the pharmacopoeial standard for thyroid; in studies on the iodization of the thyroid *in vivo;* for the comparison of the physiological activity of the thyroids, both normal and abnormal, of various animals and of various derivatives of the thyroid, and of other iodin compounds.

Before considering some of the results obtained with the test it is desirable to discuss briefly the *method* of *performing* the *test*, and some of the precautions necessary to secure satisfactory results.

It is desirable to have mice of uniform stock, of approximately the same age, preferably young adults, and also of the same sex, but by far the most important precaution is to have the mice upon a uniform diet during and for some time before the test; with this single precaution good results have been obtained with mice obtained from ordinary commercial sources. Most of my experiments, however, have been made with mice bred in the laboratory.

It is of course necessary to incorporate the thyroid in some food with which a very uniform mixture can be made; for this purpose I have found some form of cracker dust or powdered dog bread very satisfactory. Other ingredients such as casein, dried milk, etc., may be added to this and then a dough made by the addition of water or fresh milk, and the dough cut into small cakes and dried. It is very easy to collect the uneaten portion of this diet and so determine the food-intake. It is advantageous to vary the basal diet according to the season for, as stated above, mice kept upon the same diet show marked seasonal variations in their resistance to the nitril. It is desirable to have mice which have a medium degree of resistance to the drug; if the resistance is very low the absolute differences between the fatal doses of the nitril are comparatively small and the chances for error correspondingly greater. On the other hand if the resistance is very high very large doses of the nitril are necessary and in this case another factor comes in: as the dose of the nitril is increased the narcotic action of the poison becomes more and more pronounced and obscures to some extent the more specific action of the drug. Since I had found that the resistance of mice varied within very wide limits with the diet it was possible to select basal diets (to which the thyroid was to be added) which would give any desired degree of resistance. In this way the varying resistance resulting from the season could be completely overcome. In many of my recent experiments I have used a special brand of dog bread; in the late summer and autumn this was fed alone; later when the

resistance to the nitril was increasing, varying amounts of milk or cod liver oil were added. Of course the absolutely necessary condition is to have the basal diet exactly the same as the diet to which the thyroid is added; when this precaution is rigidly adhered to a wide variety of basal diets can be used.

In many of my earlier experiments I used cracker dust, mixed with water, for my basal diet; this gave a medium degree of resistance, the fatal doses throughout the year ranging only from 0.22 to 0.64 mgm. per gram mouse. In some cases, however, milk instead of water, was used in making the cakes; the results were the same except that the resistance of the mice fed on the milk cakes was lower.

The only objection, and this was of a purely theoretical nature, which has been made to a simple cracker dust diet, was that of Miura (1922 b) who suggested that the amino acids of the added thyroid proteins might supplement the "rather inadequate proteins of the wheat flour." It seems a very improbable assumption that the amino acids of from 0.5 to 5 mgm. of thyroid added to from 8,000 to 80,000 parts of cracker dust would exert any effect within the 5 to 10 days of the experiment. There is nothing to suggest that the amino acids of the thyroid are so peculiar that they would exert an action different from that of other animal proteins and I had found that the addition of the latter to the cracker dust in amounts equal to or many (sometimes hundreds of) times greater than that of the thyroid had either no or only a very slight effect and then usually in the direction of diminution of the effect of the thyroid. Among the products rich in animal proteins tested in this way was the blood and blood serum of a variety of normal animals, of animals which had received large doses of thyroid per os; casein; peptone; "nutrose;" kidney, brain, thymus; spleen; placenta; umbilical cord; guinea pig embryos, etc.[15] And, further, in many experiments in which the activity of different preparations of thyroid was compared, the gland was fed in equal amounts; in such cases any supposed supplementary action of the thyroid proteins would be the same in all cases.

Miura, however, considered it important that the experiments should be repeated using "complete diets of known nutritional value in order to obviate the possibility of a supplementary action of the thyroid proteins" and used a diet containing 22 per cent of butter fat. In conjunction with Doctor Renshaw I had repeated several years ago some of my experiments using a number of "adequate" diets including one like that of Miura. Although I obtained with the latter results similar to my earlier ones there were a number of irregularities;

[15] Of the various glands, tested aside from thyroid, only prostate, testes, mammary glands and ovaries (Hunt 1907 b, 1910) caused an increased resistance and then only when fed in amounts from 10 to 300 times greater than those in which thyroid was fed and even then the effects of the latter were more marked. It is of interest to note in this connection that Macht (1919) (1920) and Hegner (1922) found prostate to hasten the metamorphosis of tadpoles in somewhat the same way thyroid does. Rogoff and Rosenberg (1922), however, expressed doubts as to whether a specific action of this kind, analogous to that of thyroid, should be attributed to prostate.

in some series one thyroid was more, in some less, active than another thyroid. I concluded that such a diet was not very suitable, partly because it seemed more difficult to secure a uniform distribution of the thyroid in the food and partly, perhaps, for the following reason: I had found that fats markedly and quickly lowered the resistance of mice to acetonitril and I was not convinced that the opposing actions of the thyroid and of the fats, especially when fed in such large amounts as 22 per cent, were parallel: that is, it seemed possible that whereas the effect of the thyroid was persistent, the effect of the fat was of shorter duration and that the result of an injection of the nitril might be determined in part by the relation between the time of the last feeding and the time of injection. Miura's results were very irregular and I am not at all satisfied with the explanation he offers for them.

Before the appearance of Miura's papers I had commenced repeating, for another purpose, a number of my experiments, using basal diets different from those of my earlier experiments; I used these diets not because I saw any serious objections to my cracker dust diet, but rather for the sake of convenience. In the experiments reported below the basal diets consisted in most cases of a special dog bread (upon which alone mice made fully as good if not better gains than they did on Miura's diet) or of this to which 5 per cent of dried milk or 1 per cent cod liver oil or both had been added; the latter were added so as to render the resistance of the mice more uniform at different seasons.

It is of course essential to have in every series of experiments an adequate number of controls, i.e., mice receiving the basal diet without the thyroid. Failure to observe this simple and self-evident precaution has led some workers to doubt the reliability of the test.

The feeding of the thyroid was continued for from 2 to 14 days, depending upon the purpose of the test. In most of my experiments a period of about seven days was chosen; with the preparations which I used the maximum effect was reached at about this time (see table 2).

The acetonitril, dissolved in water, was then injected subcutaneously and the minimal fatal dose determined. It is necessary to employ a very pure specimen of the nitril; some of the commercial preparations are wholly unsuitable.

a. Review of previous work with the acetonitril test for thyroid. Before reporting new work and discussing an objection which has been made to the test the results (amplified in some cases by recent experiments) which have been obtained in the past will be briefly reviewed.

The reaction as a test for the presence of thyroid. Chemical methods for the detection and estimation of small amounts of thyroid are not yet available and it is necessary to resort to biological tests. The biological tests most useful, at least for most purposes, are the acetonitril test and the test based upon Gudernatsch's observations (1912) on the effects of thyroid upon the growth and metamorphosis of tadpoles. So far as I can judge, the results obtained with the tadpole test have been, in general, similar to those obtained with the acetonitril

method, but the latter seems to have the advantages of a more definite end-point (cf. Abderhalden and Schiffmann, 1922), and greater accuracy as to dosage and to be more specific;[16] it can be used at any season, and, to judge from the published results, requires less material. The latter is an important consideration when only very small amounts of material are available.

Cameron and Carmichael (1920) have recently suggested that the decrease in the rate of growth of rats caused by thyroid may be used as a test for this substance; this also requires much more material and time and it is doubtful whether as accurate results could be obtained with it.

It was early found that a milligram of active desiccated thyroid mixed with 40,000 times its weight of cracker dust, etc., could as a rule be detected by the acetonitril reaction; it was necessary under similar conditions to use many times as much of the thyroid to secure even a qualitative chemical test for iodin and this gave no information as to whether the iodin was present in the form of combination peculiar to the thyroid gland. No iodin compound other than that contained in, or derived from, the thyroid gland has been found which gives a reaction which can be confused with thyroid in a properly performed acetonitril test (Hunt and Seidell, 1910).

This test proved of value for example (Hunt and Seidell, 1908 a) in detecting thyroid in various "anti-fat remedies," "obesity foods," certain "fruits," and other nostrums in which small amounts of thyroid were mixed with large amounts of bread crumbs, tamarind paste, liquorice powder, poke root, etc., and also in certain "ethical proprietaries" advertised to physicians as combinations of other glandular organs, nucleins, etc.[17]

---

[16] Diiodtyrosin for example is stated by Romeis (1922) to be comparable to thyroid in its activity upon very young tadpoles whereas a positive acetonitril test with it has been reported only when it was injected subcutaneously in relatively enormous doses (Wuth 1921); its action apparently does not differ from that of many other iodin compounds and there should be no difficulty in distinguishing it from thyroid (cf. Hunt and Seidell, 1910).

[17] Recently Doctor Mendelsohn of Boston brought me some capsules with the request that I examine them for the presence of thyroid. I obtained a markedy positive acetonitril reaction. Doctor Mendelsohn subsequently sent me the following history of the case which led him to suspect the presence of thyroid in the capsules notwithstanding the physician's denial that they contained this drug:

"Mrs. L.—Age 32. Occupation—Housewife. Patient who had previously enjoyed good health, has been complaining for two weeks of general itching of skin and shortness of breath on exertion. The symptoms were gradually becoming worse. Physical examination showed a rapid pulse—130. Area of cardiac dullness considerably enlarged to the left: a fine muscular tremor; moderate edema of the legs and ankles; no evidence of any skin lesions. Further questioning of the patient elicited the information that she had been taking capsules for two months to reduce weight. During the past two weeks she had been given larger capsules because of the poor results from the previous medication. At the same time she was assured that these capsules were perfectly harmless and would cause loss of weight without resort to dietetic treatment. In all,

The test also proved of interest in a study of the *relation* of the *iodin* content of the *thyroid* to the *physiological activity* of the latter and in the establishment of pharmacopeial standards for the drug thyroid. At the time these studies were begun (1904) there was much uncertainty as to how much importance should be assigned to the usual presence of iodin in the thyroid. This uncertainty was due in part to the confusion of two distinct problems: *1*, the rôle of iodin for the normal functioning, and also structure, of an animal's thyroid, i.e., of the necessity or usefulness of the iodin for the animal's own well-being; and *2*, (the phase in which I was especially interested) the importance of iodin in thyroid when the latter was used as a drug, i.e., when it was to be administered to another animal or to man. The fact that the amount of iodin present in the thyroid of normal, healthy animals and man varied widely, or even seemed to be absent in some cases, led leading biochemists of that time to regard the iodin in the thyroid as a more or less accidental constituent, analogous for example to the traces of copper usually found in the liver. (For a review of the literature, see Hunt and Seidell, 1908 b.)

Marine and his co-workers made a careful study of the first of these problems, viz., the relation of the iodin to the histological structure of the thyroid; they found that a certain minimal iodin content was essential for the normal structure of the gland, but that when this was present a very considerable additional amount of iodin could be present without it having any discernible effect upon the appearance of the gland or on the animal's well-being. This additional iodin was regarded as storage iodin, making a "factor of safety." But as Seidell and I (1908 a) remarked, "it is evident that studies of this character have not a very direct bearing on the subject now under discussion, namely, the use of thyroid as a drug."

The presence of this additional iodin, which has no apparent effect upon the animal itself, led to the unwarranted assumption that it has no effect upon the activity of the gland when fed. As a matter of fact, there was already available at that time some evidence, open to criticism, however, and not in any sense quantitative, that there is a relation between the physiological action of thyroid as a drug and its iodin content. This evidence had, however, been considered inconclusive[18] and suggestions that pharmacopeial preparations be standardized

---

the patient had taken about fifty of these larger capsules in the two weeks preceding my examination. The physical signs were very suggestive of thyroid intoxication.

Clinical diagnosis: Hyperthyroidism and cardiac insufficiency caused by thyroid medication.

Subsequent history: Two weeks after discontinuance of the capsules the muscular tremor had disappeared; the pulse was 84; the area of cardiac dulness much reduced; the edema of lower extremities, while still present, was much less than before."

[18] Meltzer (1907), for example, admitted that there is a relation between the iodin content and physiological activity but maintained that the former was dependent upon the latter, i.e., that the thyroid contained more iodin because it was more active and could combine with more iodin; Mendel (1900) had expressed a similar view, stating that the variations in the iodin content may be

by their organically combined iodin content rejected both in the United States and in foreign countries; attempts at standardization were being made on the relation of the water content of the fresh gland, its size, the amount of ash, or nitrogen, etc. The final test, however, was the clinical test: different preparations were tried until one was found which gave satisfactory results. Much time was thus lost and in most cases there was no guarantee that the next lot of the same firm would be satisfactory.

Seidell and I examined a large number of thyroids, including those of a number of different species of animals, and found with rare exceptions a close parallelism between their physiological activity and iodin content. (The few exceptions were thyroids, apparently abnormal, which contained very small amounts of iodin.) In fact it soon became easy for one of us by comparing the physiological activity of a new sample with that of one of known iodin content to predict fairly accurately the percentage of iodin which would be found by the other by chemical methods; this was possible even when the thyroid was mixed with large amounts of potassoim iodide or many organic iodin compounds.

I have recently again compared the iodin content and the physiological activity of a large number of thyroids[19] (beef, sheep, hog) and have failed to find a single case in which this parallelism did not hold, although the percentage of iodin varied from 0.028 to 0.531 (see tables 4, 5, 6, 8, 11).[20] In making these comparisons, I have continued to make use of the methods described in earlier papers (1908 a, b); that is, the effects of equal amounts of the different samples and the effects of different amounts of the samples containing, however, equal amounts of iodin[21] were compared and finally the amounts of the different prep-

"due to corresponding variations in the accompanying active groups to which the iodin is perhaps attached merely as a factor of secondary importance." If such were the case thyroid low in iodin but of high physiological activity should at least occasionally be found but this has not been done.

[19] I am greatly indebted to Dr. Atherton Seidell for these preparations and for the iodin determinations.

[20] The conclusions expressed in this paper are based upon experiments on over two thousand mice; the results were so perfectly uniform and concordant that the experiments are given only in abstract. Occasionally a thyroid gave anomalous results; this was probably due to an unfortunate selection of mice, or to failure to secure a uniform mixture of the thyroid with the diet, for when the experiment was repeated (as it was at least twice in such cases) the results were found to be in harmony with the others.

[21] Miura states that I "fed thyroid in equi-iodin dosage in only one case." This statement is wholly erroneous; as the published protocols of my experiments show, I did this repeatedly and, as stated, discussed it in the text. In fact Miura seems to have completely overlooked several of my publications on the thyroid including the principal one (Hygienic Laboratory Bulletin no. 47) although he quotes papers in which frequent reference is made to these; apparently he was familiar with only a preliminary abstract of my work.

arations which gave the same degree of protection were determined. It is important to remember that there is only a limited range of dosage where there is a direct relation between the effect and the amount of thyroid: when thyroid is added in increasing amounts to the basal diet there is at first no discernible effect, then a period of minimal effect followed by a short range in which there is a direct relation between the dose and the effect, and finally, as the dose giving the maximum effect is approached, each addition has less and less influence. These considerations explain why it is not permissible to conclude that because the fatal dose of the nitril is twice as large when one thyroid is fed as when another is fed that the former is necessarily twice as active as the latter. In order to obtain accurate comparative figures it is necessary to determine the relative amounts of the different preparations which afford the same degree of protection.

It should also be remembered that the acetonitril method, like nearly all biological assay methods, is not as accurate as chemical methods; its chief value is in detecting larger differences in activity.

After Seidell and I had found that the acetonitril reaction is a very specific test for the iodin in the special form of combination in which it occurs in the thyroid and had failed to find any other iodin compound which gave a reaction which could possibly be confused with thyroid and, further, had found that there is a close parallelism between the percentage of iodin in the gland and the physiological activity in this respect, and in view of the evidence for such a relation already available, we suggested in 1907 that thyroid preparations may be standardized by the amount of iodin in organic combination.[22] The following year Marine and Williams (1908) also spoke of the desirability of there being a pharmacopeial standard for thyroid but they did not suggest an iodin standard; they seemed to have had in mind rather some method by which hyperplastic thyroids, which Marine (1907) had stated to have a poisonous action,[23] might be excluded. They did, however, report two experiments which showed that a hyperplastic

[22] About this time Doctor Osler remarked to one of us that he obtained much better results in the treatment of cretinism and myxedema with one brand of thyroid tablets than with another; our analyses showed the former to contain about four times as much iodin as the latter and to have about four times the protective action against acetonitril. Mayerle (1910) reported these tablets to be especially active in reducing the weight of dogs; he ascribed this action to the high N content of the tablets which he interpreted as meaning a high content of thyreoglobulin; he considered the latter and not iodothyrin to be the active agent.

[23] The view that hyperplastic thyroids have a toxic action does not seem to have been confirmed. Lussky (1912) found the toxic effects of normal glands to be greater than those of hyperplastic ones.

thyroid with only 0.0292 per cent iodin, when fed to a dog for 18 days caused no loss of weight, whereas a similar amount of a normal gland containing 0.1092 per cent of iodin fed to another dog caused a marked loss of weight. There was a marked accumulation of iodin in the thyroid in the second case but not in the first. The objection might be made that the thyroid fed had simply served as a source of iodin thus increasing the activity of the dog's own thyroid and that the loss of weight was due to this and not to iodin-rich thyroid acting as a more powerful drug.

Seidell and I continued our studies on thyroid as a drug and as the result of the examination of a large number of samples on the American market made exact recommendations for a United States Pharmacopoeia standard not only as regards iodin percentage, but as to the method for determining it, suggested standards for ash and moisture content, etc.[24] These standards were adopted in their entirety by the Pharmacopoeia Revision Committee and Doctor Seidell was asked to prepare the pharmacopeial test. How important it was to have such a standard may be judged from the fact that we had found the iodin percentage in some of the tablets most widely used at that time to vary from 0.084 to 0.32 and the physiological activity to vary accordingly; we had remarked that "the use of such preparations would seem to be as illogical as would that of preparations of Fowler's solution showing variations of 400 per cent in arsenic content." (Later we found samples of desiccated thyroid, which had it not been for the pharmacopeial standard might have come on the market, the iodin percentage of which ranged from a little over 0.02 to over 0.5 per cent; the latter showed about 25 times the physiological activity of the former.)

Romeis (1915) proposed the use of the tadpole test as a means for standardizing commercial thyroid preparations. He found great variations in the activity of the latter and stated that the results agreed with experiments on dogs and clinical trials. He did not, however, compare the activity of the different preparations with their iodin content. Lenhart (1915) reported that the effect of thyroid upon tadpoles was proportional to the quantity fed or to the percentage of iodin in the gland but did not attempt quantitative comparisons. Rogoff (1917) also proposed the use of the tadpole method as a means of standardizing thyroid preparations. He found commercial preparations to differ widely in activity but apparently worked with only three of known iodin content; two of these were active in proportion to their iodin percentage whereas the third showed a very low degree of activity in relation to its iodin content. Apparently, however, this latter preparation was of doubtful origin, perhaps even of doubtful authenticity.

Jensen (1920) suggested the use of the axolotl for the standardization of thyroid preparations. Jensen stated that the effect is not parallel to the iodin content

[24] Seidell alone and in coöperation with Fenger (and with the assistance of a number of manufacturers) published a number of papers on methods of assay, the seasonal variations in the iodin and ash content and other questions involved in the fixing of a pharmacopoeial standard.

I have given this history in some detail for totally erroneous accounts have been published as to the circumstances leading to the adoption of a U. S. P. standard for thyroid.

but in the only abstract of his papers which I have seen comparisons were reported only for four preparations of iodothyrin; Seidell and I several years ago reported that in some samples of iodothyrin much of the iodin is present in loose combination and is inactive. Jensen also found other organic iodin compounds to affect the metamorphosis of axolotl.

Cameron and Carmichael (1920) found a parallelism between the activity of different preparations of thyroid (with the exception of thyroxin) in causing decrease in the rate of growth in rats and rabbits and their iodin content. Seidell and I had reported somewhat similar experiments, with similar results, in 1908 but concluded that a study of the effects of thyroid upon the resistance of animals to acetonitril and other poisons to be better adapted to quantitative work.

We also used the acetonitril reaction in connection with the question of the activity of thyroid iodized *in vivo*. It was well known at that time that the thyroid proteins can take up an additional amount of iodin both *in vitro* and *in vivo* but there was much difference of opinion as to whether either or both of these products showed the physiological activity characteristic of thyroid. Apparently only one experiment had been reported indicating that the activity of thyroid iodized *in vivo* was increased, viz., that of Roos, who had found that the excretion of nitrogen was increased by the feeding of dog's thyroid the iodin content of which had been increased by the administration of large doses of potassium iodide over a period of eighteen days. The work was in no sense quantitative and different interpretations were placed upon the results; thus it was argued that the long-continued action of the iodide had simply served as a stimulus to the thyroid, increasing its activity and incidentally leading to a greater absorption of iodin. Seidell and I, in 1907, found that within twenty-four hours after the administration of 1 gram of potassium iodide to dogs the iodin content of the thyroid was increased and that its physiological activity, as tested by the nitril method, was also increased. Marine and Rogoff (1916) corroborated these results by the use of the tadpole test; they further found that when the iodide was given intravenously the thyroid showed a well-marked increase in activity in even a shorter period (sixteen to twenty hours), but that after thirty hours only a small fraction of the iodin taken up had been transformed into the specific hormone. In recent experiments on this subject (see table 10) I found that all of the iodin which had been taken up by the thyroid after two doses (1 gram) of iodoform at twenty-four-hour intervals had been completely transformed into "active" iodin when the glands were removed twenty-four hours after the second dose.

Koch (1913) used the acetonitril reaction to determine at what stage in the hydrolysis of thyroid proteins the iodin became "inactive."

I also showed (1910) by means of the nitril test that the character of the diet has much influence upon the ability of the thyroid to take up iodin and to convert it into a physiologically active form; some diets greatly facilitate such action whereas others prevent it. So far as I know this is the only instance in which it has been shown that diet influences an organ of internal secretion. The substances having this effect are as unknown as are the "vitamins."

I found only one or two (and they were apparently abnormal) thyroids among those examined several years ago which contained "inactive" iodin.[25]

On the other hand, so-called "iodin-free" thyroid, by which was meant thyroid with which not even a qualitative test for iodin could be obtained when 1 gram was examined by the Baumann method, gave a positive nitril reaction but relatively very large amounts were necessary. Such thyroid probably contained traces of iodin but it is not very probable that the amount present was sufficient to account for the physiological action.

Romeis (1922) has recently reported experiments on tadpoles from which he draws the same conclusion I had drawn, viz., iodin-free preparations of the thyroid have the typical thyroid action but that this is very greatly increased when iodin is present in some special form of combination. Schenk (1922) has reported characteristic effects upon metabolism from the adminstraton of a thyroid preparation which is stated to contain only traces of iodin; the effect was less marked than with iodin-containing thyroid.

Miura (1922b) has recorded a number of experiments which suggest that some thyroids may contain considerable amounts of "inactive" iodin;[26] I have performed many experiments with adult thyroids strictly comparable to those used by Miura as regards iodin content, species,

[25] The terms "active" and "inactive" iodin as used in this connection refer to the effect of iodin in the thyroid in increasing the resistance of mice to acetonitril. How great is the difference between "active" and "inactive" iodin may be illustrated by comparing the activity of potassium iodide with that of thyroid: potassium iodide if given in sufficient dosage affords a small degree of protection against the nitril (probably by action on the thyroid). Seidell and I (1910) concluded, from experiments in which the minimum effective doses and the degree of protection from effective doses were compared, that the iodin in thyroid is fully 10,000 times as active as that in potassium iodide. This shows what a delicate test the acetonitril reaction is for iodin in the form of combination peculiar to thyroid. This subject will be discussed further under thyroxin.

[26] The author's statements as to the relation of the iodin content of thyroid to its physiological activity seem to be inconsistent. Thus in his "Summary" he states that "the thyroid seems to be efficient in proportion to the amount of iodin contained" but in the text he speaks of how "markedly" the thyroids vary in their protective action when fed in equi-iodin amounts.

and season at which the glands were collected and found a close parallelism between their activity and iodin content.

The most marked discrepancies found by Miura between physiological activity, as tested by the nitril test, and iodin content was in the case of fetal glands. Thus fetal calf thyroids containing 0.06 and 0.28 per cent iodin afforded no protection when fed in amounts containing as much iodin as adult thyroids which afforded marked protection; when fed in ten times such amounts one of the fetal thyroids was very active. In table 10 are shown the results I obtained with three fetal thyroids[27] very similar to those used by Miura. The activity of two normal fetal thyroids containing 0.21 and 0.26 per cent iodin did not differ materially from that of normal adult thyroids when fed in equi-iodin amounts. The hyperplastic fetal thyroid containing only 0.05 per cent iodin had a low degree of activity. It is interesting to note in this connection that some normal adult thyroids with as low or lower percentages of iodin had a degree of activity closely proportional to the iodin content (see tables 4, 5, 8). This fetal thyroid seems to be one of those pathological glands which, although capable of absorbing iodin, is unable to convert it into "active" iodin.

A series of experiments was also performed with portions of thyroid removed at operation from cases of *exophthalmic goiter;* there has been much speculation as to the manner in which the iodin of the thyroid is combined in this condition; Seidell and I several years ago had found that there was a parallelism between the iodin percentage and the physiological activity; the present experiments show that the parallelism is fairly exact. As table 9 shows, the iodin percentage ranged from 0.044 to 0.587; when fed in equi-iodin dosage the degree of protection did not vary greatly except in one case. Excluding the latter the average fatal dose of the nitril was 1.08 mgm. per gram with variations of 0.22 mgm. above or below. The fatal dose of the nitril for mice fed on a normal hog thyroid (equi-iodin content) was 1.1 mgm. The amounts of thyroid fed varied (according to their iodin percentage) from 0.336 to 4.55 mgm. per day, a variation of over 1300 per cent. The thyroid which failed to afford any protection was tested in another experiment (table 10); it not only afforded no protection but seemed to lower the resistance to the nitril. This is the only instance in which I have

[27] These thyroids were obtained from the same source from which Miura obtained his specimens, the Research Laboratory in Organotherapeutics of Armour & Company; I am greatly indebted to Dr. Frederic Fenger for these preparations.

obtained such a result; unfortunately it was not possible to repeat the experiment. This preparation had the lowest percentage of iodin (0.044) but this was not much less than that of another gland in the series (0.051 per cent) which had an activity proportional to its iodin content. Moreover, as the tables show, other thyroids with iodin percentages almost as low, or lower, also had an activity corresponding closely with their iodin content. Hence it seems as if this gland, like the fetal thyroid mentioned above although capable of absorbing iodin was unable to convert it into the form of combination peculiar to the thyroid (or perhaps was unable to retain the iodin in this form). Unfortunately no histological data on the above thyroids were available and it is not known which would be considered most characteristic of Graves' disease.

Prolonged *stimulation* of one *cervical sympathetic* of the cat (by which the pupil was kept dilated for hours) did not cause any difference in the activity of the thyroid on that side as compared with that on the other; it has been reported, but not confirmed by others (see Van Dyke, 1921), that such stimulation causes a diminution in the iodin of the corresponding lobe of the thyroid. The cats in my experiments had not shown the reaction described by Levy (1916): an increased sensitiveness to epinephrin following the stimulation of the cervical sympathetic. Similar negative results were also obtained when sodium iodide had been administered before the stimulation of the sympathetic.

b. Experiments with thyroxin. The only serious criticism[28] which has been made to the acetonitril test for thyroid (that is as a test for thyroid gland substance) is that of Trendelenburg (1910). Trendelenburg found that the blood of three of four thyroidectomized cats protected mice to a marked degree against acetonitril; he interpreted this result as showing that in the absence of the thyroid toxic substances accumulate in the blood and that it is these which are responsible for the test. He explained my results with the feeding of thyroid (which he confirmed) as due to the presence of toxic substances which had been absorbed by the gland. He interpreted my experiments with iodothyrin

[28] Miura has stated that "Hunt went so far as to say that this protective action of the thyroid (towards acetonitril) is a specific physiological test for this tissue. . . . . The results were confirmed by Trendelenburg . . . . the work of Carlson and Woelfel, Olds and Lussky seems to refute the claims of Hunt." This is incorrect: the work of Carlson and Woelfel and of Lussky confirmed my claims as to the test for thyroid tissue; that of Trendelenburg seemed to refute them. The work of Olds had nothing whatever to do with the acetonitril test.

in a similar manner; I had found some samples of iodothyrin to give a markedly positive nitril reaction whereas others were almost inert; Trendelenburg suggested that some of the toxic substances which had been taken up by the thyroid had remained with the former but had been removed from the latter preparations. I had found the iodothyrin which gave a weak test to contain inorganic iodin; I think my suggestion that such iodothyrin was inactive because the iodin was not in the natural combination much more probable.

It was thought that light might be thrown upon this subject by experiments with thyroxin for it seemed very improbable that if this substance gave the reaction this could be attributed to toxic substances absorbed from the blood. It was also desired to compare the activity of this compound with that of thyroid.

During the course of these experiments Miura's paper, dealing in part with the same problem, appeared. Miura records six experiments in which thyroxin was fed. In the first one (table II) the thyroxin was only slightly active and far less active than four adult thyroids tested in equi-iodin doses; in the second experiment (table III) it was also almost inactive; in the third experiment (table IV), in which it was fed in three different amounts, it showed a low degree of activity; in the fourth experiment (table V) it was very active but was not compared with an adult thyroid; in the fifth experiment (table VI), in which it was fed in two different doses, it was active but less active than thyroid; in the sixth experiment (table VII) it was more active than a thyroid fed in more than three times the iodin dose. Thus in the nine tests thyroxin showed a very low degree of activity in five and a high or moderately high degree of activity in four. In six of the seven comparisons with adult thyroid, thyroxin was less active than thyroid when fed in equi-iodin doses; in the seventh, in which it was fed in a dose with a much smaller amount of iodin, as compared with the thyroid, it was more active. Miura attached more importance to this one comparison than to the other six; in this way he brought his results in harmony with Kendall's views as to "active" and "inactive" iodin in the thyroid. As will be shown, my results agree with Miura's six comparisons rather than with his one.

Miura attributed the low degree of protection, produced by thyroxin in several of his experiments to overdosage; if this is the case the diet must have contributed to the result for I have fed thyroxin in twice the largest dose used by Miura and for over two weeks and obtained a very high degree of protection. In my first work on the acetonitril reaction I fed thyroid containing 45 times the amount of iodin contained in the largest doses of thyroxin used by Miura and obtained high degrees of protection.

In my experiments I have used three commercial preparations of thyroxin: one the pure crystalline compound and two lots of tablets; the results were the same with all. Ten of the 0.2 mgm. tablets were found on analysis to contain 1.48 mgm. iodin (theoretical 1.3 mgm.).

Thus the tablets contained slightly more thyroxin than stated or the thyroxin contained slightly more than the theoretical amount of iodin. The activity of these samples of thyroxin was compared with that of eighteen preparations of desiccated thyroids (sheep, beef or hog); the iodin content of these ranged from 0.028 to 0.531 per cent. In addition comparisons were made with the thyroids of dogs in which the iodin content had been increased by the administration of various iodin compounds and with the thyroids of eight cases of exophthalmic goiter.

The thyroid, or thyroxin, was incorporated into basal diets which varied somewhat in the different series. The duration of the feeding was, except in special cases, from six to nine days.

In other experiments the thyroxin was injected intravenously and the effects compared with those of thyroid and thyroxin administered per os.

The purpose of these experiments was twofold: *1*, to determine if thyroxin when fed to mice has an action qualitatively and approximately quantitatively like that of thyroid; this was to test Trendelenburg's hypothesis that the acetonitril reaction is not caused by the gland substance per se but by substances absorbed by it from the blood; and *2*, to determine if thyroxin, in proportion to its iodin content, differs in the degree of its activity from thyroid. As the tables of experiments (tables 2 to 9, 11 and 12) show, thyroxin has the qualitative action of thyroid; its quantitative action also is vastly greater than that of any other iodin-containing substance except the thyroid. But in not a single case was the thyroxin, in proportion to its iodin content, as active as any of the normal adult thyroids. As the protocols show, the thyroids in proportion to their iodin content were about one and a half times as active as the thyroxin; it required, for example, 0.003 mgm. and 0.0045 mgm. iodin in thyroxin to afford the same degree of protection as 0.002 mgm. and 0.003 mgm. in thyroid. Moreover, the variations in the activity of the different thyroids in equi-iodin doses when compared with one another and with thyroxin were slight; there is nothing to suggest that ordinarily thyroid contains iodin "inactive" as regards the acetonitril test.

In no case did I obtain the marked discrepancies which Miura reported, although I used thyroids containing approximately the same percentage of iodin and also preparations containing much less as well as much more than he did.

Cameron and Carmichael (1921), using as a criterion the retardation

in the rate of growth of rats, found thyroid to be from two to four times as active as thyroxin when fed in equi-iodin dosage. They suggested that the thyroxin was destroyed in the intestine to a greater extent than was the thyroid. A similar assumption could be made to explain my results; I endeavored to test this by comparing the activity of thyroxin, when injected intravenously into mice every day for a week or more, with thyroid and thyroxin when fed. The results of these experiments are shown in table 12. It will be seen that although the protective action was developed more rapidly when the drug was given intravenously than when fed, the degree of protection ultimately attained was not materially greater; thyroxin containing 0.003 mgm. iodin and administered intraveneously was about as active as thyroid fed in doses containing 0.002 mgm. iodin and distinctly less active than thyroid containing 0.003 mgm. iodin.

Thus as far as the highly specific and characteristic acetonitril reaction is concerned, thyroid is more active than thyroxin containing an equal amount of iodin. As stated above, Miura obtained similar results in six of the seven experiments he reports but attributed the results to overdosage; Cameron and Carmichael also obtained similar results in their feeding experiments but explained them on the basis of underdosage (due to an assumed destruction of the thyroxin in the intestine). My intravenous injection experiments as well as the feeding experiments show that thyroid is more active than thyroxin.

Several hypotheses in explanation of this might be suggested; one is that the thyroid gland contains a compound more highly iodized (and more active) than thyroxin and that some of this iodin (which is perhaps in a more labile form than the iodin in thyroxin) is split off in the preparation of the latter. As is well known, Kendall found two types of iodin compounds in the products of the hydrolysis of thyroid; one was practically inactive physiologically whereas the other represented the thyroxin. It seems not impossible that in the tissue itself the former was in a combination more active than it is in thyroxin. This raises the question whether the action of thyroxin represents the full physiological activity of the thyroid; it does not seem to do so as regards the acetonitril reaction which of course is simply one phase of the physiological action of thyroid (and which may prove to be as true a measure of metabolic activity as the usual clinical methods) or in the effects upon growth (which are also probably connected with a fundamental action of the gland). This is of more theoretical than of practical interest from the standpoint of the use of these agents as drugs; appar-

ently thyroxin will give all the desired effects of thyroid and has the great advantage of uniformity and of being capable of administration by other than the oral route (besides representing the most notable work yet done on the chemistry of the thyroid).

The question whether thyroxin really represents the active secretion of the thyroid is, however, of fundamental importance in studies on the physiology and pathology of the gland. But my experiments have no bearing upon this question; they relate only to the condition of the iodin in the gland itself, not with what the gland secretes.

The statement that part of the iodin in thyroid is "inactive" seems to be based entirely upon the fact that when thyroid is hydrolyzed by caustic soda only a part of the iodin can be isolated as thyroxin. I know of no experiments, other than those with acetonitril and those on growth, in which the physiological activity of thyroid has been carefully compared with that of thyroxin. Koch (1913) found that a series of hydrolysis products of the thyroid showed a gradual decrease in activity (as tested by the acetonitril method) per unit of iodin. His "iodothyrin" fraction for example showed per unit iodin from one-half to three-fourths the activity of the thyroid. This is not far from the relation I found between the activity of thyroxin and thyroid; it may be that thyroxin constitutes the active group of the iodothyrin fraction rather than of thyroid itself.[29]

Kendall (1919a), in one of his latest publications, states that the proportion of "inactive" and "active" iodin (as obtained by the aqueous alkaline hydrolysis of fresh thyroid) is "remarkably constant" during different seasons and in different species; he suggests that this may represent a condition of equilibrium between inactive iodin on its way to be converted into active iodin (thyroxin) and the thyroxin but perhaps it represents the opposite: that in the hydrolysis of thyroid a

[29] The statement, based upon a publication of Pick and Pineles (1909), is sometimes made that iodothyrin is "not even a concentrated form of desiccated thyroid." But as I pointed out several years ago (1913) the work of Pick and Pineles throws no light on this question for they compared the effects of about 20 parts of thyroid with those obtained from the iodothyrin from one part of thyroid, that is, their relative dosage was about 20 times greater in the former case. Some of the pioneer work on the effect of thyroid upon metabolism was done with "iodothyrin" and Magnus-Levy (1907) concluded that the action of iodothyrin was identical with that of thyroid by every criterion for thyroid activity but that it seemed to be somewhat weaker. The same is shown by the acetonitril reaction: iodothyrin at its best (some of the commercial preparations contain loosely bound iodin) seems to have about the degree of activity of thyroxin.

constant percentage of the active iodin is split off yielding a more stable but less active compound (thyroxin).

TABLE 2

In the following table are shown the effects of feeding thyroxin and a hog thyroid in equi-iodin amounts; the fatal dose of the nitril for the mice receiving the thyroid was about one and a half times as great as that for the thyroxin-fed mice

PREPARATION FED	PER CENT OF IODIN	MGM. SUBSTANCE FED DAILY	MGM. IODIN FED DAILY	FATAL DOSE MGM. PER GM. MOUSE
Control: "Special dog bread"				0.35
Thyroxin	65.0	0.003	0.00195	2.2
Hog thyroid 696	0.44	0.44	0.00194	3.4

TABLE 3

The thyroid used in the above experiment was later compared with thyroxin upon a different lot of mice; about the same relations as to activity held. The results also show a summation of the effects of thyroid and thyroxin

PREPARATION FED	PER CENT OF IODIN	MGM. SUBSTANCE FED DAILY	MGM. IODIN FED DAILY	FATAL DOSE MGM. PER GM. MOUSE
Thyroxin	65.0	0.0015	0.00975	[illegible]8
Hog thyroid 696	0.44	0.22	0.0097	2.7
A mixture of above		0.0015 + 0.22	0.00975 + 0.0097	More than 3.4

TABLE 4

The above thyroid was compared with that of another species which contained only about one-ninth as much iodin

PREPARATION FED	PER CENT OF IODIN	MGM. SUBSTANCE FED DAILY	MGM. IODIN FED DAILY	FATAL DOSE MGM. PER GM. MOUSE
Thyroxin	65.0	0.00153	0.001	1.8
Thyroxin	65.0	0.00307	0.002	2.8
Hog thyroid 696	0.44	0.228	0.001	2.5
Hog thyroid 696	0.44	0.455	0.002	4.2
Beef thyroid 664	0.049	2.04	0.001	2.2
Beef thyroid 664	0.049	4.08	0.002	4.0

The constancy in the relation of the iodin containing constituents found by Kendall in the products of hydrolysis of the thyroid at different seasons and in different species is what would be expected from my findings that the activity of thyroid, when fed in equi-iodin doses does not vary with season or species or (except in the rare cases noted above) with the percentage of iodin in the gland.

TABLE 5

In the following experiment three beef thyroids containing widely different percentages of iodin were fed

PREPARATION FED	PER CENT OF IODIN	MGM. SUBSTANCE FED DAILY	MGM. IODIN FED DAILY	FATAL DOSE MGM. PER GM. MOUSE
Control (dog bread + 1 per cent cod liver oil).....				0.48
486	0.028	5.36	0.0015	1.6
486	0.028	10.71	0.003	4.2
665	0.071	2.11	0.0015	1.2
665	0.071	4.22	0.003	3.4
674	0.426	0.352	0.0015	1.2
674	0.426	0.704	0.003	3.6
Thyroxin	65.0	0.00232	0.0015	1.0
Thyroxin	65.0	0.00464	0.003	2.2

TABLE 6

Three preparations of hog thyroid containing different percentages of iodin were compared with each other and with thyroxin in three different amounts

PREPARATION FED	PER CENT OF IODIN	MGM. SUBSTANCE FED DAILY	MGM. IODIN FED DAILY	FATAL DOSE MGM. PER GM. MOUSE
515	0.133	2.256	0.003	4.0
687	0.213	0.705	0.0015	2.3
687	0.213	1.410	0.003	4.1
698	0.444	0.338	0.0015	2.2
698	0.444	0.676	0.003	4.2
Thyroxin	65.0	0.00232	0.0015	1.0
Thyroxin	65.0	0.00463	0.003	3.6
Thyroxin	65.0	0.00695	0.0045	4.0

TABLE 7

The activity of crystallin thyroxin was compared with that of thyroxin tablets and with that of a hog thyroid. The smaller doses of thyroxin were apparently near the lowest effective doses and the degree of protection was slight. The largest dose of the hog thyroid was probably super-maximal. Thus in neither case were the doses in the range where there is a direct relation between the amounts of the drugs fed and the effect. The results show, however, that the two samples of thyroxin had the same degree of activity and that this was less than that of the hog thyroid

PREPARATION FED	PER CENT OF IODIN	MGM. SUBSTANCE FED DAILY	MGM. IODIN FED DAILY	FATAL DOSE MGM. PER GM. MOUSE
Controls (dog bread + 5 per cent dried milk + 1 per cent cod liver oil).....				0.57
Thyroxin cryst.....	65.0	0.00232	0.0015	0.75
Thyroxin cryst.....	65.0	0.00461	0.003	2.7
Thyroxin tablets.....	65.0	0.00232	0.0015	0.7
Thyroxin tablets.....	65.0	0.00461	0.003	2.5
698, hog.....	0.444	0.338	0.0015	3.00
698, hog.....	0.444	0.676	0.003	4.5

TABLE 8

The thyroids of different species and containing widely different percentages of iodin were fed in equi-iodin amounts. Although the amount of thyroid fed varied from 0.565 to 10.71 mgm. (nearly 1900 per cent) the degree of resistance produced was approximately the same in all with one exception: no. 478 seemed distinctly weaker than the others. In other experiments (see table 10) this thyroid did not show an anomalous result; the mice receiving this thyroid in this experiment may have been less resistant or there may have been some error. The fatal dose for the thyroxin-fed mice was relatively low; probably the amount fed was below the range where there is a direct relation between the amount fed and the effect

PREPARATION FED	PER CENT OF IODIN	MGM. SUBSTANCE FED DAILY	MGM. IODIN FED DAILY	FATAL DOSE MGM. PER GM. MOUSE
Controls (dog bread + 5 per cent dried milk + 1 per cent cod liver oil.....				0.55
496, beef.....	0.028	10.71	0.003	2.5
468, sheep.....	0.042	7.14	0.003	2.5
665, beef.....	0.071	4.23	0.003	2.5
515, hog.....	0.133	2.26	0.003	2.3
687, hog.....	0.213	1.41	0.003	2.2
658, beef.....	0.216	1.38	0.003	2.3
474, sheep.....	0.235	1.27	0.003	2.6
478, beef.....	0.335	0.894	0.003	1.7
501, hog.....	0.531	0.565	0.003	2.6
Thyroxin.....	65.0	0.00463	0.003	1.2

TABLE 9

Thyroid from cases of exophthalmic goiter

PREPARATION FED	PER CENT OF IODIN	MGM. SUBSTANCE FED DAILY	MGM. IODIN FED DAILY	FATAL DOSE MGM. PER GM. MOUSE
Controls (dog bread + 5 per cent dried milk + 1 per cent cod liver oil).....				0.3
1*	0.044	4.55	0.002	0.5 or less
2	0.051	3.92	0.002	1.1
3	0.159	1.26	0.002	0.9
4	0.189	1.06	0.002	1.2
5	0.191	1.058	0.002	1.2
6	0.329	0.61	0.002	1.0
7	0.43	0.465	0.002	1.3
8	0.587	0.336	0.002	0.85
Thyroxin	65.0	0.00307	0.002	0.65
Thyroxin	65.0	0.00463	0.003	1.0 or less
687, hog	0.213	0.942	0.002	1.1

* Compare table 10.

These findings of Kendall as to the constancy of the composition of thyroid as regards the iodin-containing constituents are an argument for the value of the pharmacopoeia method of standardization, whatever view is taken as to the occurrence of "active" and "inactive" iodin in the thyroid.

TABLE 10

A variety of thyroids, normal and abnormal, were fed in this experiment. The thyroid marked "dog 5" was obtained by mixing the thyroids of 9 dogs each of which had received two doses of 1 gram of iodoform on two successive days; the thyroids were removed 24 hours after the second dose. The iodin percentage in the thyroids of a group of similar dogs which had received no iodoform was 0.218 whereas that in the iodoform group was 0.358. The thyroids marked "dog 6" and "dog 7" were obtained in a similar manner: the dogs in "7" had received no iodoform whereas those in 6 had received iodoform in the manner described above. Although the percentage of iodin had been increased by the administration of iodoform the results show that it had become fully "active" within 48 hours. The thyroid from one of the fetal calves (A_2, hyperplastic) had little protective action, whereas those of the other two (normal) were almost as active as those of normal adults

PREPARATION FED	PERCENT OF IODIN	MGM. SUBSTANCE FED DAILY	MGM. IODIN FED DAILY	FATAL DOSE MGM. PER GM. MOUSE
Controls (dog bread + 5 per cent dried milk + 1 per cent cod liver oil)				0.7
501, hog	0.531	0.377	0.002	1.6
501, hog	0.531	0.474	0.0025	2.0
501, hog	0.531	0.565	0.003	5 or more
Dog 5	0.358	0.697	0.0025	1.8
Dog 6	0.277	0.905	0.0025	2.1
Dog 7, normal	0.131	1.91	0.0025	2.0
A_3 large fetal calf	0.05	5.0	0.0025	0.8
A_4 normal fetal calf	0.21	1.19	0.0025	1.9
A_5 normal fetal calf	0.26	0.963	0.0025	1.6
Exophthalmic goiter I*	0.044	5.67	0.0025	0.38
478, beef	0.335	0.745	0.0025	2.0

* Compare table 9.

TABLE 11

In the experiment recorded below a number of mice of approximately the same weight (12 grams) were placed on the diets indicated. Beginning on the fourth day of the feeding one or more mice was injected with acetonitril. The data are incomplete but they show that a marked degree of protection is present in some cases on the fourth day and that this is not much increased after the fifth or sixth day; that the larger the amount of iodin (in thyroid) fed the more rapid the development of the protection; that thyroxin fed in amounts containing 0.001 mgm. iodin caused only a low degree of protection after 12 days; that thyroxin containing six times this amount of iodin caused a much greater degree of protection; that 2 samples of thyroid containing respectively 0.028 and 0.531 per cent of iodin caused approximately the same degree of protection when fed in amounts containing equal amounts of iodin although the absolute amount of thyroid fed was nearly 19 times as great in the former as in the latter case; that these samples of thyroid were as active as thyroxin fed in amounts containing one and a half times as much iodin. (These latter comparisons are less conclusive than those in some other experiments reported for the doses fed were so large that the maximum protection obtainable with any dosage was approached; that the maximum effect was not quite reached is shown by the greater degree of protection afforded, at least in the earlier days of the experiment, by a larger dose of thyroxin.) The mice on the basal diet became less resistant as the diet was continued although they gained steadily in weight; there was a corresponding diminution in the resistance of the thyroxin and thyroid-fed mice

Diets (and amounts of thyroid preparation and iodin fed daily) and fatal doses of acetonitril in milligrams per gram

○ = Recovered + = Died

DAY OF FEEDING	BASAL DIET DOG BREAD 94, DRY MILK 5, COD LIVER OIL 1	THYROXIN 0.00154 MGM. 0.001 MGM. I	THYROXIN 0.00694 MGM. 0.0045 MGM. I	THYROXIN 0.00926 MGM. 0.006 MGM. I	BEEF THYROID (0.028 PER CENT I) 10.71 MGM. 0.003 MGM. I	HOG THYROID (0.531 PER CENT I) 0.565 MGM. 0.003 MGM. I
4	0.38 +	0.41 +	0.5 ○	0.7 ○	1.0 ○	1.3 ○
			1.0 ○	1.3 ○	2.0 ○	2.6 ○
5	0.33 +	0.38 +	2.0 ○	2.6 ○	3.2 ○	3.7 ○
		0.33 ○	3.3 ○	3.7 ○	3.4 ○	3.8 ○
6	0.28 +	0.70 +	3.8 ○	4.1 ○	3.8 +	4.2 +
7	0,23 ○	1.0 +	4.2 +	4.4 ○	4.0 +	4.4 +
8	0.26 ○	0.7 +	4.1 ○	4.8 +	3.8 ○	4.2 ○
9	0.3 ○	0.5 ○	4.3 +	4.6 +	4.1 ○	4.3 +
			4.2 +	4.4 +	3.6 ○	3.8 ○
10	0.33 +	1.0 +	4.4 +	4.7 +	4.3 +	4.2 +
11		0.7 +				
12	0.29 +	0.6 +	3.8 +	4.1 +	4.3 +	4.3 +
13	0.25 +	0.5 ○				
14	0.22 ○	0.55 ○				

TABLE 12

In this table the effects of daily intravenous injections of thyroxin upon the nitril resistance are compared with those of the feeding of thyroxin and of thyroid containing equal as well as greater and smaller amounts of iodin. The mice were of approximately the same weight (14 grams)

Amounts of thyroxin and thyroid per mouse fed or injected intravenously daily; also fatal doses of acetonitril in milligrams per gram

DAY OF INJECTION OR FEEDING	CONTROLS ON BASAL DIET; DOG BREAD 94, DRIED MILK 5, COD LIVER OIL 1	THYROXIN INTRAVENOUSLY 0.00463 MGM. (0.003 MGM. I)	THYROXIN FED		THYROID 501 (HOG) (0.53 PER CENT I)	
			0.00463 mgm. (0.003 mgm. I)	0.00695 mgm. (0.0045 mgm. I)	0.376 mgm. (0.002 mgm. I)	0.565 mgm. (0.003 mgm. I)
	0.25 ○	1.0 ○	1.0 +	0.8 ○	1.0 +	0.6 ○
1	0.60 +					1.5 ○
		1.5 ○	0.8 ○	1.8 ○	0.8 ○	2.0 ○
2	0.4 +	2.2 ○	1.2 +	2.5 +	1.2 ○	2.5 ○
		2.5 ○	1.1 ○	2.3 ○	1.8 ○	3.0 ○
3	0.32 +	2.8 +	1.5 ○	2.8 ○		
		3.0 ○	2.0 ○	3.5 ○	2.5 ○	3.6 ○
4	0.27 ○	3.5 +	2.5 ○	4.0 +		
5		3.2 ○	3.0 ○	3.8 +	3.2 ○	4.1 ○
6	0.3 ○	3.6 ○	3.6 +	3.7 ○	3.6 ○	4.6 +
		4.1 +				
7		3.8 ○	3.3 ○	3.6 ○		
8	0.32 ○	4.1 +	3.7 ○			

3. THE ACETONITRIL REACTION AS A TEST FOR THYROID SECRETION AND IN CONNECTION WITH GRAVES' DISEASE. Although I have repeatedly stated that the acetonitril test was developed in connection with a study of the thyroid gland when used as a drug, its possible value as a test for the secretion of the gland has aroused more interest. Seidell and I (1910) stated: "we have never made positive claims for this reaction as a test for thyroid secretion; we claim that when properly and critically applied it is an extremely delicate test for thyroid gland (including iodothyrin) and one by which this can be detected with more certainty than by any other method at present known. We do believe, however, that there is considerable evidence, somewhat indirect

it is true, that it is a test for thyroid secretion as the latter occurs in the body;" we then referred to some of the conditions under which we believed that it could probably be used as a test for thyroid secretion.

Although I am at present investigating this question, a brief discussion of its present status may be of use in removing certain misapprehensions.

Carlson and Woelfel (1910) attempted to detect thyroid secretion in the lymph from hyperplastic dog thyroids by this test. The results were negative as it seems to me was to have been anticipated even assuming that these glands were forming an active secretion and that this reached the circulation through the lymph. Seidell and I suggested that Carlson and Woelfel could at best have fed scarcely more than the equivalent of 0.02 to 0.01 mgm. thyroid daily—an amount from 5 to 10 times too small to be detected by the test even under the most favorable conditions.

Lussky (1912) attempted to detect thyroid active principle in the blood of animals and man after the administration of the gland, or of their extracts, by mouth or by intravenous or intraperitoneal injection; I had already reported negative results in experiments when the gland was administered per os to guinea pigs and Ghedini (1911) had published negative results with the blood of a man to whom thyroid had been fed. Lussky considered his results also to have been negative. Yet a close examination of his protocols suggests that he may have obtained slightly positive results in some cases; it would hardly seem logical to expect marked results when it is considered how promptly foreign substances are removed from the blood.

Carlson and Woelfel argued that if the acetonitril reaction is a test for t[h]yroid secretion thyroidectomized animals should show an altered resistance to the poison; Lussky performed such experiments upon gray mice and obtained no change in the resistance. I had already reported similarly negative results in normally fed white mice and guinea pigs. But I had considered that these results had little bearing upon the question of the value of the reaction as a test for thyroid secretion. For there was no evidence that the thyroids removed were producing a secretion;[30] the animals in neither my own nor Lussky's experiments showed any effect of the thyroidectomy.

I thought that if the thyroid were made hyperactive by some means and then removed the animals might show a changed resistance which might reasonably be attributed to a lack of thyroid secretion. I used three methods for securing hyperactive thyroids: the administration of iodin compounds, the feeding of special diets and a combination of the two (1907 b; 1911). The results with guinea pigs were as follows: the feeding of thyroid to these animals *lowers* their resistance to the

[30] As Kendall (1919 b) remarked: thyroid secretion "is not fundamentally essential to life."

nitril; the administration of iodin compounds, if it has any effect at all,[31] also lowers the resistance. Thyroidectomy had little effect upon the resistance of normal animals; the administration of "Sajodin" or of potassium iodide markedly lowered the resistance of the normal animals but had no effect upon the thyroidectomized animals.

The resistance of mice to the nitril may be made to vary to an extraordinary degree by diet; I assumed, as a working hypothesis, that these results were due in part, in some cases, to effects of the diets upon the thyroid's activity. It was found that removal of the thyroids greatly diminished the effect of some of the diets. Thus groups of mice were fed for several weeks on a diet of crackers, milk and eggs; their resistance to the nitril was very low, the fatal dose of the poison being but 0.15 mgm. per gram mouse. I assumed, tentatively, that this indicated a thyroid producing little active secretion. As a matter of fact thyroidectomy had no effect upon the resistance of these mice to the nitril. Other groups of mice were, at the same time, being fed upon a diet of oatmeal and liver; these mice were extraordinarily resistant to the nitril, the fatal dose of this being 4.3 mgm. per gram. I assumed, tentatively, that this suggested a very active thyroid secretion, or perhaps a gland capable of producing a very active secretion. Removal of the thyroid in these animals reduced the resistance by one-half, the fatal dose now being but 2.2 mgm. The only explanation I can see for such a result is that the lowered resistance came about as a result of removal of the thyroid and that in cases in which the thyroid is very active the acetonitril test is a test for thyroid secretion; the latter, however, may not be secreted unless some need for it develops. (The resistance of the mice of this group was, after removal of the thyroid, still far greater than that of the mice fed on the cracker-milk-eggs diet. This may have been due to some special action of the latter in lowering the resistance or to there still being an excessive amount of thyroid secretion in the body of the oatmeal-liver-fed mice, for the tests were made twelve days after the thyroidectomy; but perhaps it is more probable that the oatmeal-liver diet had elements, perhaps sulphur compounds, which of themselves had an antidotal action to the nitril. Even with the latter assumption, however, it is clear that removal of the thyroid had had a marked effect.)

[31] The absence of an effect of iodin in some animals I attributed, as has already been discussed, to the inability of the thyroid to convert the iodin into thyroid active principle; at least one cause of the latter seemed to be the character of the preceding diet.

The thyroids of the mice which had a low degree of resistance were removed and fed to other mice whose resistance to the nitril was later tested; these thyroids, when administered as a drug, had a low degree of activity. On the other hand, the thyroids of the oatmeal-liver fed mice when fed to other mice made the latter very resistant to the poison. Thus in these cases the thyroids which produced the most active secretion in the body were also the most active as a drug.[32]

Finally a few words may be said as to the probability of it being possible to detect, by the acetonitril test, thyroid secretion in the blood in cases of Graves' disease, assuming such secretion to be present. I obtained blood from such a case at autopsy and obtained a strikingly positive reaction with it (1907c). The first tests were made with the fresh blood; the remainder of the blood was dried and has been tested a number of times since, always with striking results. It was recently retested (15 years after it was obtained) and was found to be as active as ever (see table 13). Since the blood of a number of normal animals and of man, also that of thyroidectomized animals and the serum of normal horses (and also that of horses immunized against tetanus and diphtheria toxins) did not give the test I ventured to state that this was a "probable" demonstration of thyroid secretion in the blood in a case of Graves' disease.[33] But in the same paper I cited experiments to show that, assuming that the positive acetonitril test resulted from an increased amount of thyroid secretion in the blood in Graves' disease, there is a difficulty in the way of accepting such an increased secretion as the only factor in this condition: the blood of animals to which very large amounts of thyroid had been administered did not give the reaction and I suggested that the further assumption would have to be made that in Graves' disease there is an interference with the destruction or elimination of thyroid secretion. Somewhat later I pointed out that the results could be equally well explained on the hypothesis that the substance in the blood responsible for the reaction was one which had stimulated the mouse thyroid to increased activity,

[32] It was such experiments as these which led me (1918), in another connection, to determine if the previous administration of iodides to cats would lead to a more marked effect of the stimulation of the cervical sympathetic nerve upon the reaction described by Levy (1916): an increased sensitiveness of the vascular system to epinephrin when the cervical sympathetic was stimulated; the results were negative.

[33] As I stated at the time, one of the chief reasons for publishing this note was the hope that physicians would send me blood for further tests.

or had led to an accumulation in the thyroid of active substance which could easily be excreted, rather than thyroid secretion itself; I had found that certain diets contained substances which almost certainly have such a stimulating action.

Most of those who have reported similar tests have obtained positive results. Ghedini (1911) for example applied the test to the blood of 32 patients suffering from a great variety of diseases. A positive reaction was obtained in 9 cases: three of these were typical cases of Graves' disease; in two others there was a marked enlargement of the thyroid; one was a case of *adiposis dolorosa;* the remaining three cases had chronic nephritis. Ghedini considered that there was independent evidence of thyroid hyperfunctioning in chronic nephritis (cf. Barker and Hanes, 1909). Ghedini considered the test of much value in the diagnosis of hyperthyroidism.

Carlson and Woelfel reported "clear-cut negative results" in one case.

I have had few opportunities of repeating the test; of two samples of blood tested a few years ago, one gave a positive, but not a marked, reaction and the other was negative. Little blood was available, however.

Recently, through the kindness of Doctors Porter, McIver, Sturgis, Blumgart and Grabfield, I have been able to test samples of blood from four cases of Graves' disease and also that from a few other pathological conditions and from normal individuals.

The results are shown in tables 13 and 14; in table 13 is also included the result of a retest of the sample of blood from my 1907 experiments. The test was positive in all cases. The results were especially striking in the experiments shown in table 13; in some instances the blood from the cases of hyperthyroidism protected the mice against from four to eight times the dose fatal to the controls. Blood from cases of mild nephritis, ulcerative colitis and acute middle ear (see table 14 also) had little or no effect. The results recorded in table 14 are less striking; the basal diet employed seemed less suitable for showing the differences between normal and hyperthyroidism blood. Nevertheless the blood from the three cases of hyperthyroidism gave distinctly positive results; negative results were obtained with the samples of blood of the two normal, or approximately normal, individuals. The blood of a case of chronic nephritis gave a decidedly positive reaction.

The only conclusion which I would venture to draw from these experiments, as well as from those of Ghedini, is that human blood some-

times contains unidentified substances which cause a marked increase in the resistance of mice to acetonitril; so far this effect has been reported only with blood obtained from cases of hyperthyroidism (or cases in which thyroid function was apparently disturbed) and in cases of nephritis, but very few pathological conditions have been studied.

The effect of these bloods, as far as the nitril reaction is concerned, is the same as that of thyroid (or thyroxin). But, aside from other considerations to be mentioned below, the very magnitude of the reaction would make a person hesitate to conclude that the reaction was due to thyroid secretion in the blood; as the tables show the protection caused by 0.1 gram of the dried blood was comparable with that caused by 0.003 to 0.004 mgm. thyroxin or 0.2 to 0.3 mgm. desiccated thyroid. Thus, on such a supposition, the activity of the blood of a patient would correspond to the activity of two or three grams of desiccated thyroid or to 20 or 30 mgm. of thyroxin. Such a condition might be conceivable if all of the active principle secreted remained in the blood but this is very improbable.

Then the experiments of Trendelenburg with the blood of thyroidectomized cats show that however conclusive the acetonitril reaction is as a test for thyroid gland, or for thyroxin, if the conditions are properly controlled, great caution is necessary in extending it to such a complicated mixture as pathological blood.

I have no reason to doubt the correctness of Trendelenburg's results although they have not apparently received much confirmation. Lussky reported a positive reaction with the blood of thyroidectomized rabbits but his figures are not very convincing: the difference between the effects of the blood of the thyroidectomized rabbits and that of normal rabbits (between which alone comparisons should be made), was slight. Before the appearance of Trendelenburg's and Lussky's papers I had performed similar experiments with the blood of thyroidectomized sheep and of guinea pigs;[34] the resistance was slightly lowered, as not infrequently occurs also when normal blood is fed. I have since found,

[34] Adult sheep and guinea pigs show, as a rule, few symptoms after thyroidectomy; Lussky does not state how his rabbits reacted to the operation. Trendelenburg's cats showed marked effects very promptly; Professor Straub (in whose institute Trendelenburg's experiments were performed) stated, in a personal letter, that the cats were "wild cats" which reacted extraordinarily severely to thyroidectomy. The occurrence of a marked reaction on the part of the blood may be dependent upon the severity of the symptoms resulting from the removal of the thyroids.

in one case, the blood of a thyroidectomized monkey to afford a slight degree of protection as compared with that of a normal monkey. A

TABLE 13

The experiments recorded below were performed at different periods and upon different lots of mice; the dog bread which constituted the basal diet was not the same in the different series, but was the same in each series. The weight of blood represents the amount of dried blood fed daily

	PREPARATION FED	FATAL DOSE ACETONITRIL
		mgm. per gm.
Ser. I XIII. 134	Controls, dog bread	0.42
	Hyperthyroidism, "Je," metabolism + 77, "very toxic," 0.1 gm	>3.2
	Thyroxin 0.004 mgm	3.0
II XIII. 136	Controls, dog bread	0.55
	Mild nephritis, "Zi," 0.1 gm	0.63
	Hyperthyroidism, "Em," metabolism, + 55, 0.1 gm	>2.30
	Thyroxin 0.003 mgm. (0.00195 mgm. I)	2.20
	Thyroid (0.44 per cent I) 0.44 mgm. (0.00194 mgm. I)	3.40
III XIII. 138	Controls, dog bread	1.10
	Mild nephritis, "Zi," 0.05 gm	0.78
	Hyperthyroidism, "Em," 0.05 gm	1.80
	Thyroxin 0.0015 mgm. plus "Em" 0.05 gm	3.60
	Thyroxin 0.0015 mgm. (0.00098 mgm. I)	1.90
	Thyroid (0.44 per cent I) 0.22 mgm. (0.00097 mgm. I)	2.60
IV XIII. 142	Controls, dog bread	1.10
	Ulcerative colitis, "Si," 0.1 gm	1.10
	Hyperthyroidism, "MacC," 0.1 gm	>2.20
	Cat, normal, 0.1 gm	0.92
	Cat, thyroidectomized, 0.1 gm	2.50
	Thyroxin 0.003 mgm	3.00
V XIII. 146	Controls, dog bread	0.72
	Acute middle ear, "Hu," 0.1 gm	1.10
	Hyperthyroidism "Ca I," metabolism + 35, 0.1 gm	1.60
	Above, 10 days postoperative, 0.1 gm	>2.10
	Thyroxin 0.003 mgm	>4.00
	Thyroid cat, 2 mgm	5.00

very marked positive test was obtained in the only experiment performed upon a cat (table 13). The thyroidectomized cat's weight had increased from 3.43 kilos to 3.72 kilos within twenty days after thyroidectomy, but no other effect of the operation was noted.

Thus the experimental evidence at present available indicates that the blood of thyroidectomized animals is strikingly like that of Graves' disease. But whereas the action of the latter *may* be due to an increased amount of thyroid secretion that of the former of course can not be. The action of the blood of the thyroidectomized animals may be due to some substance acting upon the nitril either directly or through its effects upon body cells; or it may be thought of as stimulating the thyroid to increased activity, or potentially increased activity. I have

TABLE 14

Some of the bloods fed in the above experiment were also fed in the following experiment; the basal diet was different; 0.1 gram dried blood was fed daily

	PREPARATION FED	FATAL DOSE ACETONITRIL
		mgm. per gm.
XIII. 182	Controls, dog bread 94, dried milk 5, cod liver oil 1	0.58
	Normal human. "Gr. II."	0.62
	Normal human (middle ear) "Hu."	0.58
	Hyperthyroidism "Sc."	1.10
	Hyperthyroidism "Em."	0.83
	Hyperthyroidism "Ca II"	0.77
	Chronic nephritis "Jo"	1.00
	Hog thyroid (0.213 per cent I) 0.94 mgm	2.00

already shown that there is evidence that there are substances in some articles of food which act in the latter way and I am now endeavoring to determine if the blood of thyroidectomized animals (and also that of Graves' disease) protects thyroidectomized mice against the nitril.

If it is shown that the same substance or substances are responsible for the reaction in both cases, some of the current theories as to the causation of Graves' disease would require modification: the primary cause of the disorder might have to be sought at least in part outside of the thyroid gland. As already stated, there are analogies for such a hypothesis: a diet of oats leads to a great enlargement of the thyroid and, as I believe I have shown, to an increased ability of the gland to form an active secretion.[35] At the same time there may be a diminution of the body's ability to destroy the thyroid secretion.

[35] Seidell and I found (and Miura confirmed us) that the substance or substances in oats having this action may be extracted with alcohol. I am continuing the study not only of the blood of Graves' disease and of thyroidectomized animals but also the study of oats and other foods which seem to have some special relation to the thyroid.

In any case the isolation and identification of the substance or substances causing this reaction may be of interest not only in connection with disorders of the thyroid but in connection with chronic nephritis (in which condition the blood at times at least gives the same reaction) and possibly in other disturbances of metabolism. The "blood chemistry" of some of the samples of blood giving the most marked effects was reported "normal;" hence the substance or substances responsible for the acetonitril reaction are apparently not among those ordinarily considered in clinical chemical work.

SUMMARY

The above experiments confirm and extend the work previously reported as to the marked effects of diet upon the resistance of mice to acetonitril; they also indicate that vitamins may be concerned in this action.

Earlier work showing a very close parallelism between the physiological activity of thyroid (as determined by the acetonitril test) and iodin content is fully confirmed; this has been found to be true for the thyroids of a number of species of animals and for thyroids with extreme ranges in iodin content; for the thyroid in Graves' disease (with one exception); for thyroid iodized *in vivo* and for fetal thyroids (with the exception of one abnormal gland).

The physiological activity of thyroxin, both by feeding and by intravenous injection, is (expressed in terms of iodin content) less than that of thyroid.

No evidence of the presence of physiologically inactive iodin in the thyroid was found (except in the case of two abnormal glands).

Additional experiments are reported showing that the blood in certain pathological conditions (especially those in which the thyroid is involved, but also in nephritis) contain unknown, or unidentified, substances which markedly increase the resistance of mice to acetonitril.

BIBLIOGRAPHY

ABDERHALDEN, E. AND O. SCHIFFMANN. (1922) Pflüger's Arch., cxcv, 157.
BARKER, L. F. AND F. M. HANES. (1909) Amer. Journ. Med. Sci., cxxxviii, 469.
BIRCHER, E. (1922) Schweiz. med. Wochenschr., lii, 713.
BURGET, G. E. (1917) This Journal, xliv, 492.
CAMERON, A. T. AND J. CARMICHAEL. (1920) Journ. Biol. Chem., xlv, 69.
CAMERON, A. T. AND J. CARMICHAEL. (1921) Journ. Biol. Chem., xlvi, 35.
CARLSON, A. J. AND A. WOELFEL. (1910) This Journal, xxvi, 32.
GHEDINI, G. (1911) Wien. klin. Wochenschr., xxiv, 736.

GUDERNATSCH, J. F. (1912) Arch. f. Entwicklungsmech., xxxiv, 457.
HEGNER, R. W. (1922) This Journal, lxi, 298.
HOPKINS, F. G. (1912) Journ. Physiol., xliv, 423.
HOPKINS, F. G. (1920) Biochem. Journ., xiv, 721.
HUNT, R. (1902) Johns Hopkins Hosp. Bull., xiii, 213.
HUNT, R. (1904) Arch. Int. de Pharm. et de Thérap., xii, 448.
HUNT, R. (1905) Journ. Biol. Chem., i, 1.
HUNT, R. (1907 a) Hygienic Laby. Bull. 33, Washington.
HUNT, R. (1907 b) Journ. Amer. Med. Assoc., xlix, 1323.
HUNT, R. (1907 c) Journ. Amer. Med. Assoc., xlix, 240.
HUNT, R. AND A. SEIDELL. (1908 a) Journ. Amer. Med. Assoc., li, 1385.
HUNT, R. AND A. SEIDELL. (1908 b) Hygienic Laby. Bull. 47, Washington.
HUNT, R. (1910) Hygienic Laby. Bull. 69, Washington.
HUNT, R. AND A. SEIDELL. (1910) Journ. Pharm. Exper. Therap., ii, 215.
HUNT, R. (1911) Journ. Amer. Med. Assoc., lvii, 1032.
HUNT, R. AND A. SEIDELL. (1911) Amer. Journ. Pharmacy, lxxxiii, 407.
HUNT, R. (1913) Forchheimer's Therapeusis of internal diseases, i, 82.
HUNT, R. (1918) This Journal, xlv, 231.
HUNT, R. (In press) In Heffter's Handbuch der exper. Pharmakol., i.
JENSEN, C. O. (1920) Compt. Rend. Soc. Biol., lxxxiii, 315; also Hosp. Tid., 63, 505 (quoted from Ber. gesammt. Physiol., 1921, v, 402).
KENDALL, E. C. (1919 a) Journ. Biol. Chem., xxxix, 125.
KENDALL, E. C. (1919 b) Endocrinol., iii, 156.
KOCH, F. C. (1907) Proc. Amer. Pharm. Assoc., lv, 371.
KOCH, F. C. (1913) Journ. Biol. Chem., xiv, 101.
LEVY, R. L. (1916) This Journal, xli, 492.
LENHART, C. H. (1915) Journ. Exper. Med., xxii, 739.
LUSSKY, H. O. (1912) This Journal, xxx, 63.
MCCOLLUM, E. V., N. SIMMONDS AND W. PITZ. (1917) Journ. Biol. Chem., xxix, 341.
MCCOLLUM, E. V. AND N. SIMMONDS. (1922) Journ. Amer. Med. Assoc., lxxviii, 1953.
MACHT, D. I. (1919) Proc. Soc. Exper. Biol. and Med., xvi, 138.
MACHT, D. I. (1920) Journ. Urology, iv, 115.
MAGNUS-LEVY, A (1907) v. Noorden's Handb. d. Path. d. Stoffw., ii, 324.
MARINE, D. (1907) Johns Hopkins Hosp. Bull., xviii, 359.
MARINE, D. AND W. W. WILLIAMS. (1908) Arch. Int. Med., i, 349.
MARINE, D. AND J. M. ROGOFF. (1916) Journ. Pharm. Exper. Therap., ix, 1.
MAYERLE, E. (1910) Zeitschr. f. klin. Med., lxxi, 71.
MENDEL, L. B. (1900) This Journal, iii, 290.
MELTZER, S. J. (1907) N. Y. med. Monatschr., xix, 223.
MIURA, M. (1922 a) Journ. Lab. Clin. Med., vii, 267.
MIURA, M. (1922 b) Journ. Lab. Clin. Med., vii, 349.
OSBORNE, T. B. AND A. J. WAKEMAN. (1919) Journ. Biol. Chem., xl, 385.
PICK, E. P. AND F. PINELES. (1909) Zeitschr. exper. Path. u. Therap., vii, 518.
PRINGSHEIM, J. (1908) Biochem. Zeitschr., xii, 143.
RENSHAW, R. R. (1921) Amer. Naturalist, lv, 73.
ROGOFF, J. M. (1917) Journ. Pharm. Exper. Therap., x, 199.

ROGOFF, J. M. AND W. ROSENBERG. (1922) Journ. Pharm. Exper. Therap., xix, 353.
ROMEIS, B. (1915) Zeitschr. f. gesammt. exper. Med., iv, 379.
ROMEIS, B. (1922 a) Klin. Wochenschr., i, 1262.
ROMEIS, B. (1922 b) Arch. f. Entwicklungsmech., l, 410.
SCHENK, P. (1922) Arch. exper. Path. u. Pharm., xcii, 1.
SEIDELL, A. (1911) Journ. Biol. Chem., x, 95.
SEIDELL, A. AND F. FENGER. (1913) Journ. Biol. Chem., xiii, 517.
SEIDELL, A. AND F. FENGER. (1914) Hygienic Laby. Bull. 96, Washington.
SEIDELL, A. (1922) Journ. Amer. Chem. Soc., xliv, 2042.
SUNDSTROEM, E. S. (1922) This Journal, lx, 397, 434.
TRENDELENBURG, P. (1910) Biochem. Zeitschr., xxix, 396.
VAN DYKE, H. B. (1921) This Journal, lvi, 168.
WUTH, O. (1921) Biochem. Zeitschr., cxvi, 237.

THE DISTRIBUTION OF THE VAGUS NERVES TO THE SINO-AURICULAR JUNCTION OF THE MAMMALIAN HEART

GEORGE BACHMANN

From the T. T. Fishburne Laboratory of Physiology of Emory University

Received for publication October 20, 1922[1]

Physiological investigations. Since the discovery in 1845 by the brothers Weber (1) of the inhibitory action of the vagus nerves upon the heart, there has grown up an extensive literature dealing principally with the details of this action. The distribution of the vagi within the heart does not seem to have enlisted the attention of physiologists to the same extent, although differences in the inhibitory power of the two vagi on the heart of the turtle were observed as early as 1869 by Meyer (2). Subsequently, other investigators working on the same heart recorded similar differences: Gaskell (3), Wesley Mills (4), Kazem-Beck (5), Guyénot (6). A more thorough study of these differences together with an explanation of their cause was made by Garrey (7). He showed that the vagi have a preponderant homolateral effect especially noticeable upon the basal veins and less well defined upon the auricles. He was able to obtain crossed effects, however, on strong stimulation. He demonstrated that the greater effectiveness of the right vagus in causing complete stoppage of the heart beat is due to its more extensive distribution to the right basal vein (precava) in which the pacemaker is located. He showed also that when the left vagus fails to inhibit the intact heart, it nevertheless induces stoppage of the left vein and auricle.

More recently Cruickshank (8) reinvestigated the question on the same heart taking the positive change of potential first observed by Gaskell (9) as the criterion of vagus action. He states that "each vagus nerve exerts its greatest effect upon its own side, and on the opposite side an effect which, generally, is definitely less than but may, in the case of the right vagus, be almost as great as that of its homolateral action." The method used necessitated prolonged stimu-

[1] A preliminary report is found in the Proc. of the Amer. Physiol. Soc., this Journal, 1922, lix, 468.

lation, and the observations consisted of a comparison of the action of the vagi on the two auricles.

Attempts to determine the distribution of the vagi in the mammalian heart, by physiological methods, are of relatively recent date. Wybauw (10) who, at about the same time as Lewis (11), located the primary seat of impulse formation in the sino-auricular node, noted that stimulation of the vagus suspends the activity of this node. Rothberger and Winterberg (12) state that the sino-auricular and atrio-ventricular nodes are both innervated by the vagi but that the vagus fibers are so mixed that their effects cannot be isolated. They were of the opinion, however, that the left vagus sends a greater number of fibers to the atrio-ventricular node. A more complete investigation of the relation of the vagi to the sino-auricular node was undertaken by Flack (13). He showed that weak stimulation of the node causes inhibition of the entire heart; that the application of atropine to the node suspends the activity of the right vagus upon auricles and ventricles; that, at times, the same effect is produced on the left nerve, while at other times this nerve, though no longer able to affect the auricles, is still capable of affecting the ventricles. He obtained virtually the same results after clamping the node, and expressed the opinion that when the left vagus is distributed to the node, it innervates its upper portion, while the right nerve innervates its lower portion. Marchand and Meyer (14) investigated the distribution of the vagi in the rabbit's heart by the nicotine method. The application of nicotine to the posterior wall of the right auricle, in the area between the left and right venae cavae, rendered both vagi inoperative. They concluded, therefore, that the ganglion cells in relation with the right and left vagus lie in common groups.

Cohn (15) and later Ganter and Zahn (16) confirmed the findings of Rothberger and Winterberg as regards the preponderating influence of the left vagus upon the atrio-ventricular node. The same relative effects of right and left vagus stimulation were shown to obtain in the human heart by Canby Robinson and Draper (17). Lewis (18) made a special study of the control of the vagi on the A-V node and came to the conclusion that both vagi are distributed to this node, and furthermore that the right vagus exercises upon it a greater chronotropic influence than the left. The same author (19), in his paper dealing with the course of the excitatory process through the dog's auricles, expresses the opinion that the main action of both vagi is upon the head of the sino-auricular node and that the right vagus must supply

the tail of the node also, inasmuch as it has a greater chronotropic influence. Lastly, Schlomovitz, Eyster and Meek (20), on the basis of excision experiments and of cooling of the nodal tissue, reached the conclusion that both vagi exercise their greatest chronotropic effect by depressing the automaticity of the head of the sino-auricular node, that both vagi have a greater chronotropic influence upon the whole of the sino-auricular node than on parts having a lesser automaticity, and that both nerves have a chronotropic control over the auriculo-ventricular node.

From this brief review of the literature, it is apparent that the distribution of the vagi in the mammal has been sought exclusively in connection with the specialized tissues of the heart, viz., the sino-auricular and the auriculo-ventricular nodes. That positive results have been obtained does not necessarily preclude the possibility that the vagi may be distributed to other areas. That the distribution of the postganglionic fibers is to be sought in the nodal tissue cannot be disputed; but that the ganglion cells around which the vagus fibers arborize are necessarily located with the nodal tissue is open to doubt. *A priori* considerations would lead one to suppose that the entire sino-auricular junction would contain ganglion cells in relation with the vagus nerves, for it is preëminently at this junction that ganglion cells have been found most abundantly in the heart of the lower vertebrates.

The sino-auricular junction is represented in the adult mammalian heart, in part, by the line of attachment of the right venous valve. This line is along the crista terminalis and the base of the Eustachian valve. In animals in which this structure is absent, the tissue above the mouth of the coronary sinus as far as the limbus of the fossa ovalis represents the line of attachment of the Eustachian valve. A portion of the sino-auricular junction must be sought also at the place of fusion of the left venous valve with the septum secundum, namely, in that portion of the limbus of the fossa ovalis that becomes identified with the tubercle of Lower. According to Keith and Flack (21), the atrio-ventricular node does not belong to the sino-auricular junction but to the junction between the auricular canal and the ventricle. Aschoff (22), however, divides this node into two parts, one part belonging to the auricle and, presumably, originating from the sinus valves, the other derived from the auricular canal. The atrial portion of the atrio-ventricular node is said to embrace the opening of the coronary sinus. It is doubtful whether this extension of the atrio-ventricular node to the sinus proper is justifiable.

Histological investigations. Vignal (23), who studied the cardiac ganglia in the principal divisions of the vertebrates, states that in the bony fishes there are two groups of ganglion cells, one group, upon the auricle, forming a complete circle around the venous canal that joins the auricle to the ventricle, the other situated along a large branch that passes from the nerve circle of the auricle to the aortic bulb. The distribution of the ganglia in the frog's heart is familiar to every physiologist and, according to Vignal, is applicable to the heart of the turtle and other reptiles. In the bird—pigeon—the same author found many small ganglia in the plexus that ramifies on the surface of the auricles beneath the visceral pericardium; he located large ganglia in the neighborhood of the pulmonary veins as well as a number of ganglia in the auriculo-ventricular groove.

Remak (24) was the first to show the existence of nerve ganglia in the heart of mammals. Schklarewski (25), who made an extensive study of the heart of mammals and birds, states that the ganglia form two closed rings. The first, at right angles to the base of the heart, follows the interauricular septum; the second, at right angles to the first, is located in the auriculo-ventricular furrow in the neighborhood of the interauricular septum where it crosses the first and anastomoses with it. According to this author, the middle of the auricular septum is free of nerves and ganglia while the greater mass of the ganglia seen in the heart of mammals is located near the opening of the superior vena cava. Dogiel (26), describing the nerves and ganglia of the dog's heart, found also that ganglia are numerous between the auricle and the opening of the superior vena cava where they spread upon this vein. He describes a second group between the opening of the superior vena cava and the root of the aorta and finally a third group between the base of the right auricle, the root of the aorta, and the pulmonic veins. All these ganglia, according to Dogiel, are on the surface of the heart, under the pericardium. Vignal (23), who investigated the heart of the rabbit, monkey—macacus sinicus—and man, mentions ganglia as accompanying the plexus that ramifies on the auricles, but states that they are more numerous in the neighborhood of the pulmonic veins.

Keith and Flack (21), in their original description of the sino-auricular node, mention the presence of nerve cells and fibers within the node; from their description it is evident that the nerve cells were not found in great numbers. These findings have been confirmed by Oppenheimer and Oppenheimer (27), by Walter Koch (28) and in greater detail by Meiklejohn (29). These authors, however, have described important

groups of ganglion cells as occurring under the epicardium, near the S-A node of the dog's heart (Oppenheimer and Oppenheimer, Koch) and of the heart of the monkey, guinea pig, rat, cat and man (Meiklejohn).

Lissauer (30), who studied adult human hearts in serial sections, found ganglion cells only in the auricles, both on the posterior wall in the part lying between the two auricles and in the posterior atrio-ventricular furrow on the right and left side. These ganglion cells, he states, lie in three or four groups under the epicardium. He quotes the work of Koplewsky, who found two groups of ganglion cells in the neighborhood of the sulcus situated between the auricles. He gives the results of Eisenlohr's and of Ott's investigations, who found ganglion cells in the auricular septum under the pericardium and in the transverse coronary sulcus. These findings are similar to those of Schwartz (31), who states that in the rat's heart there are from four to five groups of ganglion cells on the posterior wall of the auricles, located under the epicardium, more especially to the left of the interauricular septum.

Waledinsky (32), who examined the heart of the mouse, guinea pig and man, found the largest collection of ganglia in the posterior wall of the left auricle. He states, however, that ganglion cells are found also in the auricular septum, the coronary sulcus, and the mouths of the venae cavae; moreover, that all ganglia—those that lie in the septum not excepted—are found in the subpericardial connective tissue; and that there are neither ganglia nor nerve cells within the muscle tissue.

Marchand and Meyer (14) examined the rabbit's heart for the location of ganglia as a preliminary to their experiments. They found ganglia constantly in the loose connective tissue between the aorta and the pulmonary artery. Somewhat larger groups were located about the opening of the pulmonary veins. These ganglia lay fairly close under the epicardium. Ganglion cells were also regularly found in the wall of the coronary sinus. They state that a most important mass of nerve cells lies in the auricular septum; that there are two large groups, one fairly high in the septum above the fossa ovalis spreading toward the surface of the auricle in the direction of the pulmonic artery, the other lying more posteriorly in the auricular septum in a band of connective tissue that penetrates into the septum from above and behind. They state that the ganglia lying in the auricular septum and in the neighborhood of the coronary sinus spread in the immediate

vicinity of the auriculo-ventricular node, and that the main stem of the bundle and its ventricular portions were found always free of nerve cells. Morrison (33) likewise failed to find ganglion cells in the auriculo-ventricular bundle of the sheep and pig. These findings are in contradiction to those of Gordon Wilson (34) for the heart of the calf, in which he has described ganglion cells in the atrio-ventricular bundle as occurring "all the way from the coronary sinus to the distribution in the right and left walls of the ventricular septum."

The exact location of ganglionic masses in the heart, as revealed by this review of the more important histological investigations, is still uncertain. Many of the investigators have failed to be explicit concerning the location of the ganglia with reference to definite anatomical landmarks. The uncertainty of the position of ganglia in the dog's heart, in possible relation with the vagi, is such that it was deemed best to precede the physiological investigation of the distribution of the vagi to the sino-auricular junction by a histological study of this region. Accordingly, two dogs' hearts were prepared for this purpose.

Histological method. Immediately after removal of the heart from the body, a cannula was fastened in the superior vena cava, and the inferior vena cava tied. The heart was washed rapidly in normal saline and the cannula connected with a Mariotte flask filled with Müller formol and placed at such a height that the pressure of the solution was 30 cm. The heart was also completely immersed in the same solution. After 24 hours the heart was thoroughly washed in running water and the ventricles removed by a transverse section below the atrio-ventricular junction. The auricles and the superior vena cava were stuffed with cotton to preserve the normal relations and then run through ascending strengths of alcohols. When dehydrated, the auricles were trimmed in such a way that the entire interauricular septum and the median side of the mouth of the superior vena cava remained as a single block of tissue. This block was then cleared in cedar oil and xylol, infiltrated, and imbedded in paraffin. Previous to imbedding, a sketch with exact measurements of the block was made for the future placement of the ganglionic masses. The same procedure was followed in preparing the tissue containing the sino-auricular node, the block including a portion of the superior vena cava above and a portion of the auricular wall below.

The interauricular septa were sectioned serially in a frontal plane from before backward. The sections were cut 10 μ thick and every fifth section mounted and stained with thionin blue. No counterstain

was used, as the connective tissue and muscle could be discerned with sufficient clearness. The stained sections were then projected on paper with the aid of the Edinger drawing apparatus and an outline of the section, of the position of muscle, connective tissue and location of ganglia carefully drawn. With the aid of these drawings, the position of the ganglionic masses was then indicated on the sketch of the block of tissue. The identification of the ganglia was further checked with the microscope.

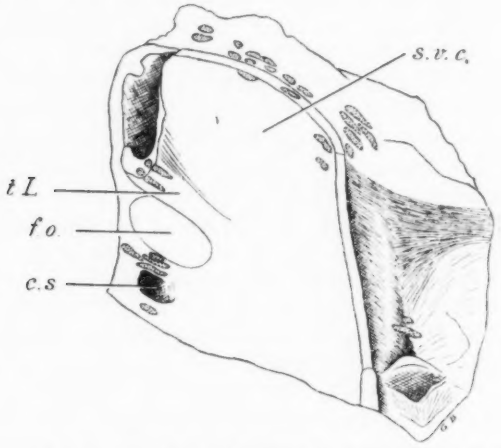


Fig. 1. Diagram of the position of ganglia in relation to the median (left) side of the mouth of the superior vena cava, the tubercle of Lower, and coronary sinus; *s. v. c.*, superior vena cava; *t. L.*, tubercle of Lower; *f. o.*, fossa ovalis; *c. s.*, coronary sinus.

Figure 1, drawn from the block of tissue, shows the location of the ganglia in the septum and on the median (left) side of the mouth of the superior vena cava. It will be noticed that a great many ganglia are found on the median side of the superior vena cava. These ganglia are immediately beneath the epicardium, in the connective tissue that overlies the muscle. The ganglia found in the tubercle of Lower are in a band of connective tissue that penetrates between the muscle masses and that probably represents the base of the left venous valve. They are a continuation of ganglia found in the fat and connective tissue situated at the angle formed by the superior and inferior venae cavae—the intercaval angle. It will be noticed also that the tissue

found between the mouth of the coronary sinus and the limbus of the fossa ovalis contains important ganglionic masses. This tissue, it will be recalled, corresponds to the base of the Eustachian valve, which together with the Thebesian valve constitutes the lower segment of the right venous valve. These ganglia are found between two masses of muscle in a band of connective tissue that apparently penetrates into the septum from a point immediately above the coronary sulcus. The muscle layer under the endocardium of the right side of the septum is thin and so the ganglia lie on the right side of the septum. Argaud (35) has described nerve fibers within the Eustachian valve and ganglia within the valve of Thebesius in the sheep and in man. It is possible that these ganglia are related to the coronary sinus group just described. Thionin stains not only the nerve cells but the nerve fibers as well. It was, therefore, possible to trace the course of a number of nerve filaments although the method is unsuited for the study of nerve terminals. Nerve fibers are abundant in the loose connective tissue above the interauricular septum on the median side of the superior vena cava; some of these fibers pass on to the postero-median side of the superior vena cava into the tubercle of Lower in the immediate vicinity of the ganglia mentioned above. Numerous nerve fibers are found along the dorsal wall of the auricles. A number of nerve fibers run in the septum from a point back of the aorta to the neighborhood of the fossa ovalis; these are probably the same fibers that have been described by Argaud as terminating in the postero-inferior portion of the interauricular septum and in the valve of Thebesius.

While nerve fibers are abundant in connection with the sino-auricular node of the dog, nerve cells are not numerous and seldom occur in such conspicuous masses as are found on the median side of the superior vena cava and in the tissue above the coronary sinus.

Having determined the exact location of the ganglia at the sino-auricular junction, a series of experiments was carried out in which the areas known to contain the ganglia were destroyed by means of an acetic dichromate solution[2] to which was added immediately before use one drop of formalin to every cubic centimeter of the solution.

GENERAL METHOD. *Preparation of the animal.* Dogs ranging in weight from 6 to 12 kilos were used. The anesthetic employed was

[2] This solution is one used in histological work as a fixing agent and consists of:

Potassium dichromate	3 grams
Glacial acetic acid	5 cc.
Water	100 cc.

ether. The vagus nerves were exposed in the neck and the thorax opened in the usual manner. Artificial respiration and etherization were maintained by means of Brodie's air warmer and anesthetizer. The stellate ganglion on each side was located, the two divisions of the ansa subclavia were freed and ligatures passed around them. Warm Ringer's solution was dropped on the heart throughout the experiment to prevent drying.

Apparatus. The miniature cardiograph devised by Wiggers (36) was used as receiving apparatus for the contractions of the right and left auricles and of the ventricles. The movable arms of the auricular myocardiographs were stitched to the tips of their respective auricular appendages. The ventricular myocardiograph was placed at the junction of the lower with the middle third of the ventricles, across the interventricular furrow, the movable arm being fastened to the left ventricular wall. This was found in preliminary experiments to be the best location for obtaining a curve of the usual contour. The contractions of these various parts were recorded by means of Frank's segment capsules and a photokymograph. The segment capsules were so turned that contraction was recorded by an upward and relaxation by a downward movement. A chronographic record was obtained by projecting the shadow of the stylus of an electro-magnetic tuning fork vibrating 100 times per second. By placing the lever of a signal magnet immediately behind the stylus of the tuning fork a centrally placed photograph of its shadow was obtained while the tuning fork was vibrating.

The inductorium used for stimulation purposes is one of Boulitte's; its secondary coil is graduated in microcoulombs or fractions thereof by means of a ballistic galvanometer.

Experimental procedure. The general procedure followed in all experiments was relatively simple. A record of the heart's action was obtained both before and after section of the vagi. That strength of current just sufficient to cause complete inhibition of the heart on stimulation of the vagi was determined and records taken. A stronger stimulus was often necessary for the left vagus and, in a number of instances, complete inhibition could not be caused on stimulation of this nerve with the strongest practicable current. The same method was used in stimulating the ansa subclavia, stronger currents than those used for the vagi being, of course, necessary to call forth an adequate response. The areas shown by the preceding histological investigation to contain ganglia were then destroyed in varying rotation by means

of the acetic dichromate formalin solution to which some insoluble carmine was added in order to permit of the subsequent location of the injection under the microscope.

The following individual areas were subjected to experimentation: the head, the tail of the sino-auricular node, the median side of the superior vena cava, the intercaval angle (head of the tubercle of Lower),

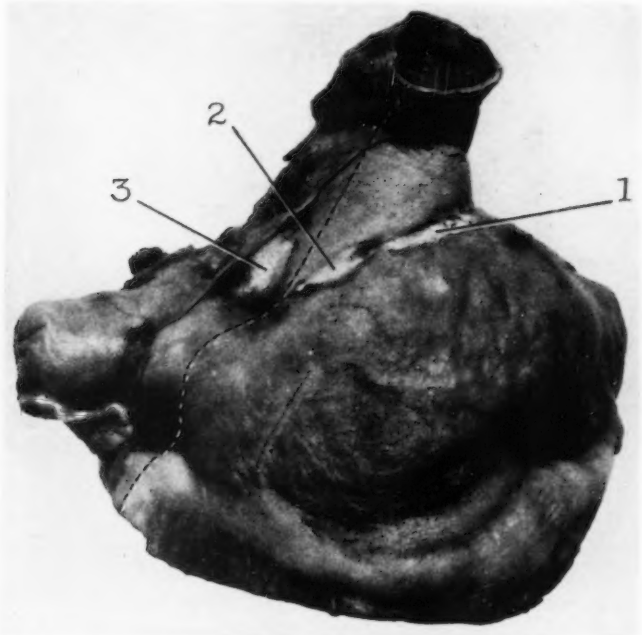


Fig. 2. Right lateral view of right auricle of dog's heart showing areas of injection. (From dog 48.) *1*, injection overlying head of S-A node; *2*, injection overlying tail of S-A node; *3*, injection of intercaval angle. The dotted line shows the line along which the auricle was split to expose the interior of the auricle shown in figure 3.

and finally the area between the mouth of the coronary sinus and the limbus of the fossa ovalis; figures 2 and 3 obtained from dog 48 show the location of the areas mentioned. All of these areas, with the exception of the last mentioned, are subepicardial. Their destruction was, therefore, easily effected by injecting the fluid under the epicardium

by means of a tuberculin syringe and a fine hypodermic needle. The coronary sinus area was reached by pushing the hypodermic needle through the apex of a funnel-like depression seen above and to the left of the coronary sinus at the point where it turns to open into the right auricle. It is apparently through this infundibular depression that the connective tissue band containing the ganglia penetrates into the septum. Records of the effects of stimulation of the vagi and of the sympathetics were obtained immediately upon destruction of each area.

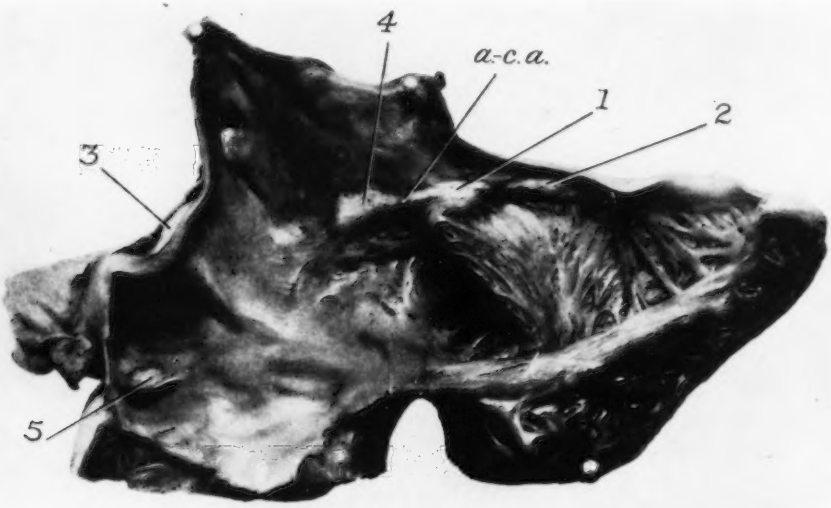


Fig. 3. Interior of right auricle showing the same areas as in figure 2, and in addition: *4*, injection of median side of superior vena cava; *5*, injection above mouth of coronary sinus. *a. -c. a.*, indicates the position of the auriculo-caval angle. Note that the injection of the intercaval angle *3* overlies the tubercle of Lower.

At the conclusion of the experiment the heart was prepared for histological study by the method already described. Immediately after fixation a sketch was made showing the exact location of the areas reached by the injection. These areas, including the surrounding tissue, were sectioned serially and every fifth section mounted and stained with alum hematoxylin and counterstained with the Van Gieson stain.

RESULTS. In analyzing the results, the chronotropic effect of the vagi on the auricles has been adopted as the criterion of vagal action. The adoption of this criterion is based on the known manner in which the vagi cause a decrease or arrest of cardiac activity, viz., by depressing the automaticity of the nodal tissue, an observation that has received ample confirmation in the course of these experiments. On the basis of this criterion, concrete expression of the effects of successive destruction of the areas known to contain ganglia can be conveyed most satisfactorily by estimating the percentile loss of inhibitory power of each vagus following this destruction. In each instance the maximum inhibitory effect obtained on stimulation of either vagus was used as the basis for computation. Table 1 shows the percentile loss for each experiment, together with other data, such as changes in A-V interval and heart rate. To facilitate the interpretation of the results obtained on the various parts of the sino-auricular junction table 2 has, furthermore, been prepared.

Relation of the vagi to the S-A node: a. Head of the node. In estimating the extent of distribution of the vagus nerves to the head and tail of the S-A node the relative percentile loss of chronotropic influence of each vagus, following the destruction of these parts of the node, was computed. There are six available observations on the head of the node. The average percentile loss—and, presumably, the average distribution of the vagi to the head of the S-A node—is as follows:

Left vagus to head of S-A node	37.22
Right vagus to head of S-A node	28.65
Difference	8.57

It will be observed from these figures that the left nerve has usually a greater influence upon the head of the node than the right nerve. The left vagus supplied this area exclusively in two cases and predominantly in three other cases. The right vagus supplied this area exclusively in one case only.

b. Tail of the node. Nine observations on the tail of the S-A node are available for computation. The average distribution of the vagi to this portion of the node yields the following figures:

Right vagus to tail of S-A node	48.53
Left vagus to tail of S-A node	31.2
Difference	17.33

TABLE 1

Effects of the successive destruction of areas of the sino-auricular junction

DOG NUMBER	TIME OF EXPERIMENT	AREA EXPERIMENTED UPON	A-V INTERVAL		RATE OF CONTRACTION OF RIGHT AURICLE PER MINUTE		PERCENTAGE OF INHIBITION WITH RIGHT NERVE BEFORE INJECTION	PERCENTAGE OF INHIBITION WITH LEFT NERVE BEFORE INJECTION	PERCENTILE LOSS OF INHIBITION OF RIGHT NERVE AFTER INJECTION	PERCENTILE LOSS OF INHIBITION OF LEFT NERVE AFTER INJECTION	REMARKS
			Before injection	After injection	Before injection	After injection					
41	3:01		0.065		222		100				
	3:02		0.065		226			40.26			Gradual lengthening of A-V interval culminating in one period of 3:1 and one of 2:1 rhythm
	3:11	Median side of superior vena cava									
	3:25:30			0.035		182				91.8	Short period of complete A-V block followed by one period of 2:1 rhythm
	3:26			0.035		182			74.68		A-V interval gradually lengthened to one period of 2:1 block
	3:28	Tail of S-A node			182						
	3:32			0.02		184			91.85		Gradual lengthening of A-V interval for three cycles followed by three periods of 2:1 A-V block
	3:32:35			0.03		184				93	A-V interval lengthened to 0.04 second toward end of stimulation
	3:34	Head of S-A node			184						
	3:37			0		187			84.5		Gradual lengthening of A-V interval for two cycles followed by one period of 3:1 block and one period of 2:1 block
	3:38			0		190				89.12	Gradual lengthening of A-V interval to 0.06 second at end of stimulation
42	3:16:30		0.07		231						
	3:19		0.07		231			41			

42	3:27	Tail of S-A node			231						
	3:30			0.07		207				74.4	Two cycles of A-V interval equal 0.08 second following onset of stimulation, then one period of 3:1 rhythm and two periods of 2:1 rhythm
	3:30:30			0.07		207				33	Gradual lengthening of A-V interval culminating in one period of 2:1 rhythm just before end of stimulation
	3:35	Head of S-A node			207						
	3:41			0		187			80.2		The rate fell gradually during stimulation to 150, culminating in a short period of complete inhibition. The A-V interval lengthened gradually to 0.085 second to return after stimulation to 0.025 second
	3:41:30			0.025		179				72.8	A-V interval gradually lengthened to fifth cycle where it was 0.075 second, then gradually decreased to 0.065 second and, in one minute, was 0. The rate fell gradually during stimulation
	3:49	Coronary sinus			179						
	3:50			0		184			95.66		A-V interval lengthened gradually (to fifth cycle) where it was 0.065 second, then gradually decreased to 0.03 second
	3:50:30			0.03		179				96	A-V interval was 0.03 second following right vagus stimulation and gradually lengthened to 0.065 second (in seven cycles) to gradually return in 30 seconds to 0
	3:54	Median side of superior vena cava			179						
	4:00			0.06		182			94		Greater length of stimulation. The decrease in rate obtained during stimulation took place gradually and required eleven cycles
	4:00:30			0.06		179				85.7	Greater length of stimulation. Decrease in rate shown was gradual and required ten cycles

TABLE 1—*Continued*

DOG NUMBER	TIME OF EXPERIMENT	AREA EXPERIMENTED UPON	A–V INTERVAL		RATE OF CONTRACTION OF RIGHT AURICLE PER MINUTE		PERCENTAGE OF INHIBITION WITH RIGHT NERVE BEFORE INJECTION	PERCENTAGE OF INHIBITION WITH LEFT NERVE BEFORE INJECTION	PERCENTILE LOSS OF INHIBITION OF RIGHT NERVE AFTER INJECTION	PERCENTILE LOSS OF INHIBITION OF LEFT NERVE AFTER INJECTION	REMARKS
			Before injection	After injection	Before injection	After injection					
43	3:13:30		0.11		193		100				Complete inhibition one cycle following onset of stimulation
	3:14:30		0.11		190			32			Complete A–V block one cycle following onset of stimulation. (Six auricular cycles without Vs)
	3:26	Coronary sinus			190						
	3:30:30			0.12		198			0		Complete inhibition one cycle following stimulation
	3:31			0.12		187				95	Four periods of 2:1 rhythm three cycles following onset of stimulation
	3:35	Head of S–A node			187						
	3:38			0.09		166			67		No change in A–V interval during stimulation
	3:40			0.09		158				91.7	A–V interval lengthened to 0.12 second during stimulation
	3:46	Tail of S–A node			158						
	3:50			0.065		156			55		
	3:51:30			0.07		139				100	A–V interval gradually lengthened to 0.12 second during stimulation
44	2:56		0.065		179		100				
	2:57		0.065		184			100			
	3:05	Coronary sinus			184						The injection was made too high in the septum, in an area posterior to the fossa ovalis
	3:09			0.065		203			0		
	3:09:30			0.065		200				0	

44	3:13	Median side of superior vena cava			200						
	3:16			0.03		176			100		No change in A-V interval during stimulation
	3:16:30			0.03		176				100	No change in A-V interval during stimulation
	3:19:30	Entire S-A node			176						
	3:21			−0.07		207			61.26		Ventricles beat before auricles. Ventricular extra systoles during stimulation
	3:25			−0.07		166				81.33	Retrograde (V-A) conduction extra systoles during stimulation
45	3:06		0.09		164		100				Complete inhibition one cycle after onset of stimulation
	3:07		0.09		164			100			Complete inhibition one cycle after onset of stimulation
	3:12	Coronary sinus			164						Injection was made too far to left of septum
	3:17			0.09		176			0		
	3:17:30			0.09		176				0	
	3:22	Tail of S-A node			176						
	3:25			0.085		174			86.2		
	3:25:20			0.085		174				100	
46	3:30		0.09		250		100				Complete inhibition one cycle following onset of stimulation
	3:30:40		0.09		250			100			Complete inhibition one cycle following onset of stimulation
	3:35	Coronary sinus			250						Injection is too high and slightly too far forward
	3:37			0.09		245			0		
	3:37:10			0.09		266				0	
	3:41	Tail of S-A node			266						
	3:42			0.085		203			93.6		No change in A-V Interval during stimulation
	3:42			0.085		203				95	Slowing occurred in last cycles of period of stimulation
47	3:14		0.08		176		100				Complete inhibition one cycle following onset of stimulation

TABLE 1—*Continued*

DOG NUMBER	TIME OF EXPERIMENT	AREA EXPERIMENTED UPON	A–V INTERVAL		RATE OF CONTRACTION OF RIGHT AURICLE PER MINUTE		PERCENTAGE OF INHIBITION WITH RIGHT NERVE BEFORE INJECTION	PERCENTAGE OF INHIBITION WITH LEFT NERVE BEFORE INJECTION	PERCENTILE LOSS OF INHIBITION OF RIGHT NERVE AFTER INJECTION	PERCENTILE LOSS OF INHIBITION OF LEFT NERVE AFTER INJECTION	REMARKS
			Before injection	After injection	Before injection	After injection					
47	3:15		0.07		176			100			Complete inhibition one cycle following onset of stimulation
	3:21	Coronary sinus			176						
	3:26			0.07		190			0		Complete inhibition one cycle following onset of stimulation
	3:26:30			0.07		187				47	Complete A–V block, 8 auricular cycles without Vs
	3:30	Head of S–A node				187					
	3:35			0.055		182			26.4		Slight inhibition four auricular cycles following onset of stimulation. Last two cycles not followed by Vs. One period of 2:1 rhythm on recovery
	3:36			0.055		182				91.2	Complete A–V block two cycles after onset of stimulation (Four cycles without Vs.) Then two periods of 2:1 rhythm
48	2:56		0.09		187.5		100				Inhibition after one cycle
	2:57		0.09		187.5			100			Complete inhibition followed by complete heart block
	3:02	Coronary sinus			187.5						
	3:05			0.095		207			0		
	3:06			0.09		207				80.2	A–V interval lengthened early in stimulation to 0.01 second but returned to 0.09 second before end of stimulation
	3:10	Tail of S–A node			207						

48	3:12			0.055		166			35.55		The decrease in rate was gradual and took place in five cycles
	3:13			0.055		166				79.6	
	3:15:30	Head of S-A node			166						
	3:20			0.06		167			0		Inhibition occurred five cycles following onset of stimulation
	3:20:30			0.06		164				79.3	
	3:25	Median side ofsuperior vena cava			164						
	3:28			0.06		171			0		Inhibition occurred five cycles after stimulation
	3:30	Intercaval angle			171						
	3:31			0.06		156			88		
50	12:17		0.105		240		100				Complete inhibition one cycle following onset of stimulation
	12:18		0.105		240			53.75			Complete A-V block one cycle after onset of stimulation (Eight auricular cycles without Vs)
	12:23	Coronary sinus			240						
	12:29			0.105		225			56.45		
	12:30			0.105		240				14	Complete A-V block one cycle after onset of stimulation (One period of five auricular cycles without Vs and one of 3:1 rhythm)
	12:34	Tail of S-A node			240						
	12:35			0.1		235			63		
	12:35:30			0.1		235				33	One period of 3:1 and three periods of 2:1 A-V rhythm
	12:41:30	Head of S-A node			235						
	12:44			0.1		214			0		Complete inhibition one cycle following onset of stimulation
	12:45			0.1		214				0	Complete inhibition one cycle following onset of stimulation
	12:50	Auriculo-caval angle and median side of superior vena cava			214						Following this injection the left auricle contracted before the right

TABLE 1—*Concluded*

DOG NUMBER	TIME OF EXPERIMENT	AREA EXPERIMENTED UPON	A-V INTERVAL		RATE OF CONTRACTION OF RIGHT AURICLE PER MINUTE		PERCENTAGE OF INHIBITION WITH RIGHT NERVE BEFORE INJECTION	PERCENTAGE OF INHIBITION WITH LEFT NERVE BEFORE INJECTION	PERCENTILE LOSS OF INHIBITION OF RIGHT NERVE AFTER INJECTION	PERCENTILE LOSS OF INHIBITION OF LEFT NERVE AFTER INJECTION	REMARKS
			Before injection	After injection	Before injection	After injection					
50	12:54:30			0.06		166			60.3		Average of two trials
	12:55			0.06		159				60	Average of two trials
51	3:53:30		0.085		166		100				Complete inhibition one cycle following onset of stimulation
	3:54		0.08		176			100			Complete inhibition one cycle following onset of stimulation
	4:02	Coronary sinus			176						The injection reached the correct area but ganglia are few, much smaller than usual and just outside the area injected
	4:18:30			0.12		200			0		
	4:20			0.12		200				0	
	4:23	Head of S-A node			200						
	4:25:20			0.075		187			72.7		
	4:26			0.075		187				76.5	
	4:29	Tail of S-A node			187						
	4:36			0.075		171			100		Rate increased to 176 immediately following stimulation
	4:37			0.075		171				100	Rate increased to 176 immediately following stimulation
52	2:17:20		0.09		200		100				Complete inhibition one cycle following onset of stimulation
	2:18		0.09		200			100			Complete inhibition one cycle following onset of stimulation

52	2:26	Median side of superior vena cava			200						
	2:30			0.07		200			0		Complete inhibition one cycle following onset of stimulation
	2:31			0.07		196				56 64	One period of 3:1 rhythm one cycle after onset of stimulation followed by ventricular rate of 111
	2:35	Coronary sinus			196						
	2:36			0.095		190			0		
	2:36:30			0.095		200				63	One period of 2:1 rhythm two cycles after onset of stimulation, then ventricular rate of 126
	2:37	Head of S-A node			200						
	2:40			0.065		190			0		Complete inhibition two cycles following onset of stimulation
	2:41			0.065		184				98 92	
	2:45	Tail of S-A node			184						
	2:46			0.065		179			80		One period of 2:1 A-V rhythm after one cycle following onset of stimulation followed by one period of 3:1 and one of 2:1 A-V rhythm
	2:47			0.06		179				100	Increase in rate during stimulation
53	3:23		0.08		182		100				Complete inhibition three cycles following onset of stimulation
	3:24		0.08		182			49 44			
	3:30	Median side of superior vena cava			182						
	3:35			0.08		176			0		Complete inhibition two cycles following onset of stimulation
	3:36			0.08		171				20 7	
	3:40	Coronary sinus			171						
	3:43			0.08		171			0		Complete inhibition one cycle following onset of stimulation
	3:44:30			0.08		167				100	

It is apparent from these figures that the right vagus exercises a greater power on the tail of the node than the left vagus. The right vagus supplied this area predominantly in four instances. In the instance in which the head of the node was supplied exclusively by the right vagus, the tail was supplied exclusively by the left nerve, and, conversely, in one instance in which the head of the node was supplied exclusively by the left nerve, the tail was supplied predominantly by the right nerve. In another instance in which the head of the node

TABLE 2

Percentile loss of chronotropic influence of right and left vagus in relation to the various parts of the sino-auricular junction

DOG NUMBER	HEAD OF S-A NODE		TAIL OF S-A NODE		MEDIAN SIDE OF SUPERIOR VENA CAVA		CORONARY SINUS AREA	
	Right vagus	Left vagus	Right vagus	Left vagus	Right vagus	Left vagus	Right vagus	Left vagus
41			17.17	1.2	74.68	91.8		
42	5.8	39.8	74.4	33.0			15.46	23.2
43	67.0	0	0	8.3			0	95.0
44					100.0	100.0		
45			86.2	100.0				
46			93.6	95.0				
47	26.4	44.2					0	47.0
48			35.55	0			0	80.2
50	0	27.0	6.55	19.0			56.45	14.0
51	72.7	76.5	27.3	23.5				
52	0	35.9	80.0	1.1	0	56.64	0	6.36
53					0	20.7	0	79.3
Averages....	28.65	37.22	48.53	31.2	43.67	67.28	10.27	49.29

was supplied exclusively by the left nerve there was a moderate predominance in the supply of the same nerve to the tail of the node. A comparison of these two computations reveals the further fact that the right vagus has a more extensive distribution to the node, taken as a whole, than the left.

Relation of the vagi to areas adjacent to the S-A node: a. The median side of the superior vena cava. There are five available observations upon the median side of the superior vena cava. The average figures obtained are as follows:

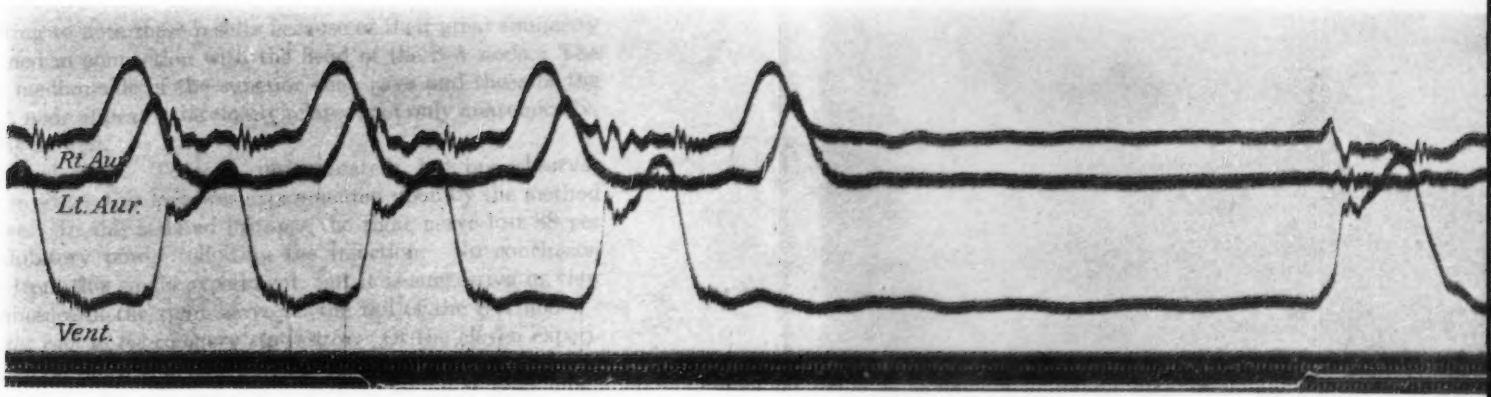


Fig. 4. Myocardiographic curves showing the effects of stimulation of the left vagus previous to experimental destruction of any of the areas conta

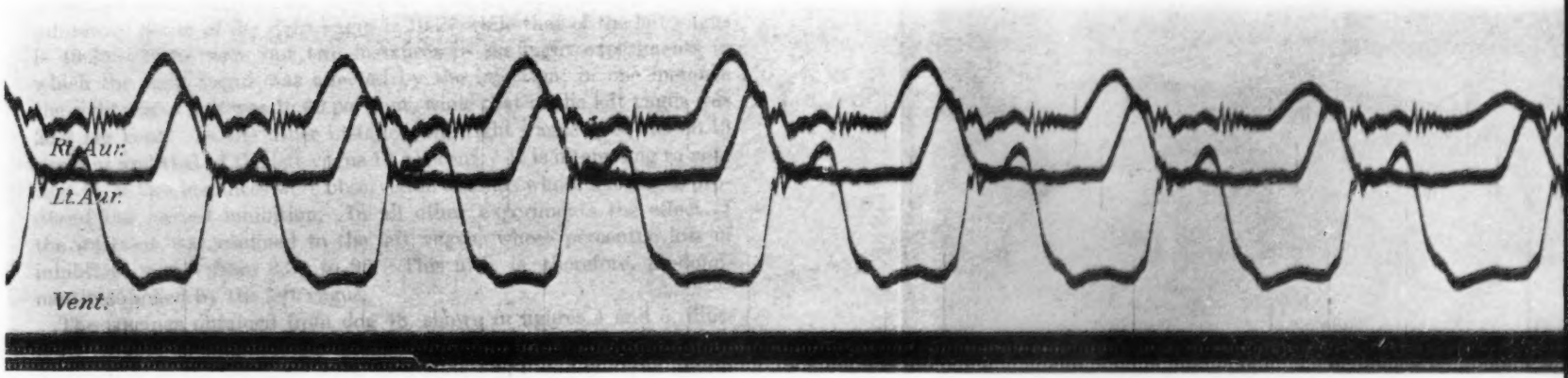
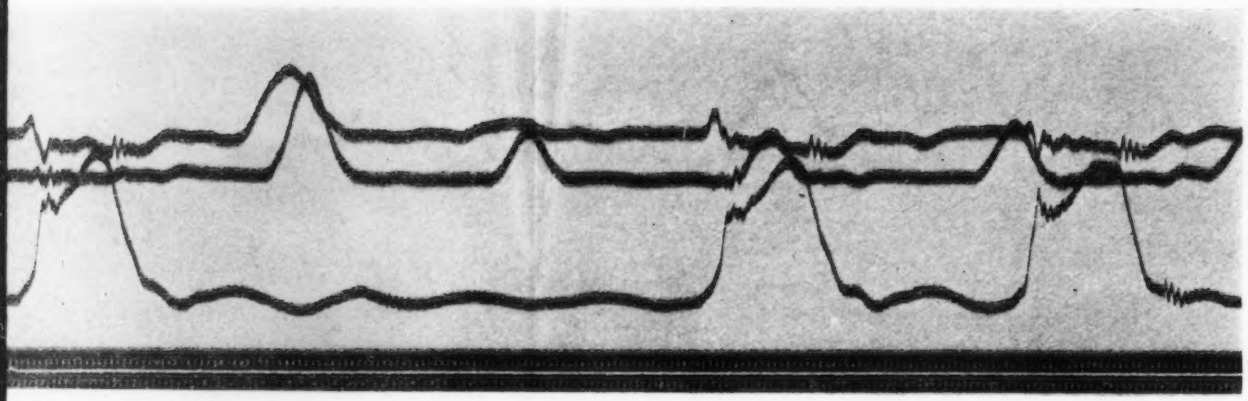
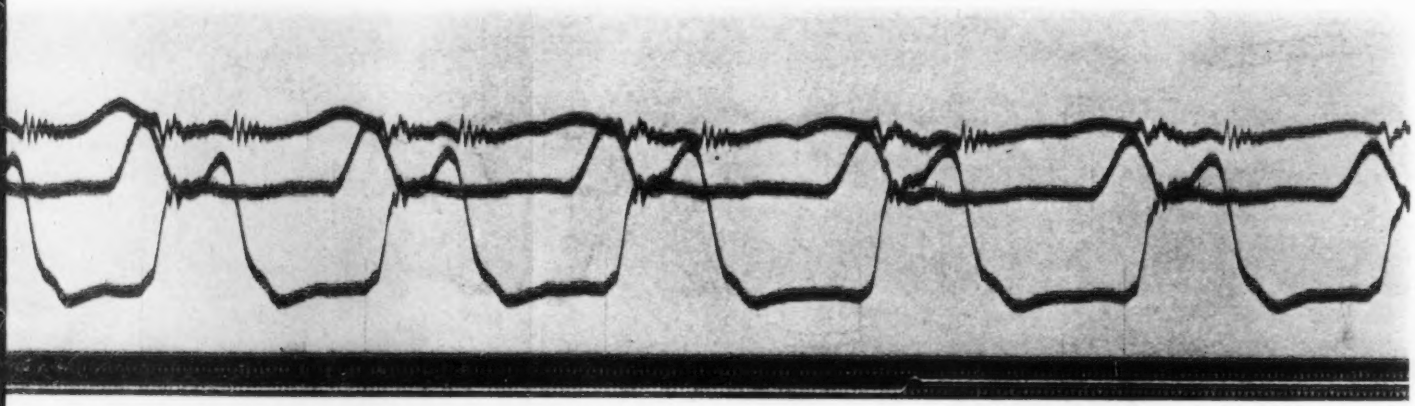


Fig. 5. Similar curves showing the effects of stimulation of the same nerve after destruction of the coronary sinus area shown i



of the areas containing ganglia. Strength of stimulation: 0.8 μQ. Duration of stimulation: 1.48 seconds. (From dog 48.)



inus area shown in figure 3. Strength of stimulation: 0.8 μQ. Duration of stimulation: 2.45 seconds.

Left vagus to median side of superior vena cava	67.28
Right vagus to median side of superior vena cava	43.67
Difference	23.61

It is interesting to note these results because of their great similarity to those obtained in connection with the head of the S-A node. The ganglia of the median side of the superior vena cava and those of the head of the S-A node appear to be closely related, not only anatomically, but functionally as well.

b. The intercaval angle. There is, unfortunately, but one observation available in which this area was experimented upon by the method described above. In this isolated instance the right nerve lost 88 per cent of its inhibitory power following the injection. No conclusion can be drawn from this single experiment, but it is suggestive in view of the predominance of the right nerve on the tail of the S-A node.

Relation of the vagi to the coronary sinus area. Of the eleven experiments performed on this area, eight were successful in that the injection was properly placed. In only one case was there no result seen. The microscopic study of this area demonstrated that but few ganglia were present and that these were smaller than usual. In all other instances a decided effect followed the injection. The average percentile loss of inhibitory power of the right vagus is 10.27 while that of the left vagus is 49.29. There were but two instances in the eight experiments in which the right vagus was affected by the injection; in one instance the right vagus loss was 15.46 per cent, while that of the left vagus was 23.2 per cent. In the other instance, the right vagus loss was 56.45 per cent and that of the left vagus 14 per cent. It is interesting to note that these two instances were observed in animals whose left vagus produced but partial inhibition. In all other experiments the effect of the injection was confined to the left vagus, whose percentile loss of inhibition varied from 6.36 to 95. This area is, therefore, predominantly supplied by the left vagus.

The tracings obtained from dog 48, shown in figures 4 and 5, illustrate the loss of inhibitory power of the left vagus consequent on the destruction of the coronary sinus area shown in figure 3. The microscopic appearance of the same area is shown in figure 6.

Discussion. Attention has already been called to the opinion of Flack (13) that the left vagus innervates the upper portion of the node and that the right nerve innervates its lower portion. Lewis, Meakins and White (19) assigned the chief seat of action of both vagi to the

head of the S-A node and deduced, from the greater inhibitory power of the right vagus, that it must likewise have an influence upon the tail of the node. Lastly, Schlomovitz, Eyster and Meek (20), on the basis of excision experiments on the upper part of the node, reached the same conclusion, although in their experiments on the effect of cooling of the same region they noted a greater effect on the left than on the right vagus.

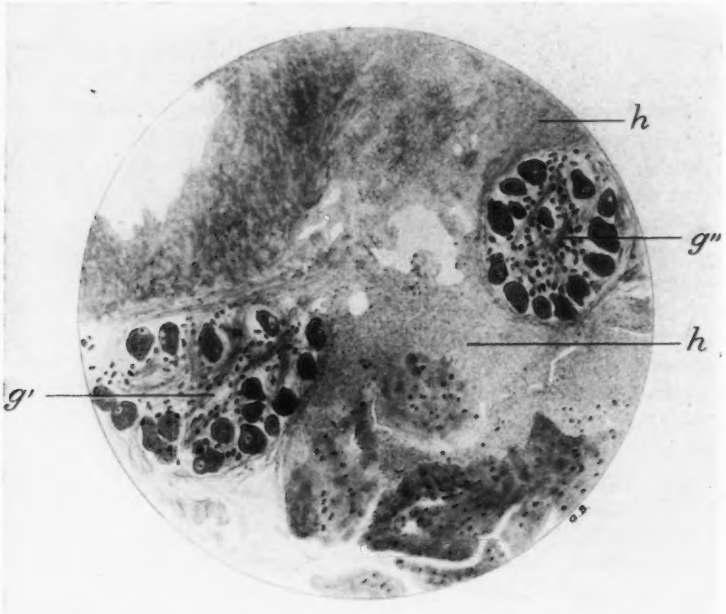


Fig. 6. Section through the coronary sinus area shown in figure 3, showing two ganglia g' and g'', surrounded by the hemorrhage, h, h, occasioned by the injection of the coagulating fluid. × 450.

The experiments on the S-A node herein reported demonstrate the fact that it is supplied by both nerves, the head of the node predominantly by the left nerve and the tail of the node predominantly by the right nerve. The right vagus predominance on the tail is, however, greater than the left vagus predominance on the head of the S-A node. Hence it is that a strength of stimulus that is sufficient to lead to a standstill of the auricles when applied to the right nerve, is often in-

adequate for this purpose when applied to the left nerve. The ability of the left vagus to produce complete inhibition seems, moreover, to be specially related to its relative distribution to the S-A node and more especially to its head. This predominance of distribution of the left vagus to the head of the S-A node offers an explanation for the observation of Meek and Eyster (37) that a shifting of the site of impulse formation to the tail of the S-A node occurs more readily on stimulation of the left vagus. A comparison of the average loss of chronotropic influence of the vagi in those instances in which both nerves produced complete inhibition with those instances in which the left nerve gave but partial inhibition shows, in fact, in the first case a decided preponderance of distribution of the left vagus to the head of the S-A node (19.2 per cent) and virtually the same percentage distribution of both nerves to the node as a whole; in the second case, a relatively less abundant distribution of the left vagus to the entire node, the percentage distribution of this nerve being 23.5 per cent less than that of the right nerve. Similarly, the average left vagus predominance on the median side of the superior vena cava is much less in those instances in which the left vagus produces partial inhibition, being but 18.9 per cent as compared with 28.32 per cent in those instances in which both vagi produce complete inhibition.

The usual effects of destruction of parts of the S-A node were noted, viz., a diminution in the A-V interval and a decrease in rate. The diminution in the A-V interval was always more pronounced on destruction of the head of the node, there being but a slight further decrease on a subsequent injection of the tail of the node. The decrease in rate of the beat was fully as great on destruction of the tail as on that of the head. Using the criterion of the shifting of impulse formation, evidence was obtained in three hearts that sinus tissue may extend to the median side of the superior vena cava. A microscopic study of this area in the hearts mentioned showed the presence of muscle tissue resembling that of the sinus in its essential histological features.

Keith and Flack (21) describe sinus tissue at the coronary sinus of the human heart "in the interval between it and the inferior vena cava and left auricle." It is reasonable to suppose that these fibers extend into the septum along the line of attachment of the right venous valve. Some observations of Zahn (38) lend support to this view. This investigator found that destruction of the sino-auricular node was not always followed by a permanent A-V rhythm; in some of his experiments the A-V interval, that had become or had approximated zero,

gradually lengthened until it became but slightly less than normal. In other experiments destruction of the S-A node caused but slight decrease in the A-V interval. In all such instances he assigned the site of impulse formation to the tissue around the mouth of the coronary sinus on the ground that the heart rate could be varied most readily by a direct application of heat or cold to that locality. The same interpretation was later adopted by Meek and Eyster (37) who used as a criterion of initial impulse formation the method of primary negativity, although they lay no claim to having placed their electrode in direct contact with the area concerned. Finally, Erlanger and Blackman (39) have shown that the tissue of the coronary sinus area possesses a high degree of rhythmicity and in some of their experiments seemed to be the seat of impulse formation. If sinus tissue exists in the coronary sinus area, particularly in relation with the ganglia found there, the destruction of this tissue should cause a shifting of the site of impulse formation to some part of the atrio-ventricular node upon destruction of the sino-auricular node. This is apparently what took place in experiment 44. In this animal the A-V interval previous to any experimental interference was 0.065 second; no change occurred following the injection of the coronary sinus area. The A-V interval became 0.03 second following the injection of the median side of the superior vena cava and became negative (-0.07 sec.) following the destruction of the entire S-A node; in other words, a V-A rhythm became established. This is the only experiment in which such a result was obtained. It is worth noting that the greater part of the coronary sinus area injected was posterior to the fossa ovalis. The coronary sinus area may well be a secondary site of impulse formation, but there is no valid reason for assigning it—as Aschoff has done—to the atrio-ventricular node; the coronary sinus area is a part of the old sinus venosus; the atrio-ventricular node, as already stated, is that part of the specialized tissues derived from the auricular canal.

The injection fluid, there is reason to believe, caused through its coagulating influence complete and permanent destruction of the tissues with which it came in contact. It abolished not only the function of the vagus mechanism contained in the areas experimented upon but also the activity of the sinus tissue. Its action is, therefore, comparable though not identical with the effect produced by cold, and the explanation of the results of cooling offered by Schlomovitz, Eyster and Meek (20) holds with at least equal force for the results here presented. The injection of a coagulating fluid is probably preferable,

however, to the application of cold because of the accuracy with which it can be placed and because of its strictly localized action. Obviously, it is not specific in its action; it acts on the nerve fibers and ganglion cells as well as on muscle. It cannot give information, therefore, concerning the relation of the preganglionic fibers of the vagi to the various ganglionic masses located at the sino-auricular junction.

A comparison of the results of destruction of the tissue overlying the head of the sino-auricular node and of the median side of the superior vena cava shows that both areas are related predominantly to the left vagus. A similar comparison between the tail of the S-A node and the intercaval angle shows that the right vagus is predominantly related to these areas. Whether these experimental results are due to the destruction of the ganglia shown to be present in each of these respective areas, can only be settled by the employment of the nicotine method of Langley (40).

THE RELATION OF THE VAGI TO THE GANGLIA OF THE S-A JUNCTION AS DETERMINED BY THE NICOTINE METHOD. *Method.* Eleven dogs were used in this series of experiments. They were anesthetized and prepared as previously explained. The contractions of the right auricle and ventricles were recorded on the Hürthle kymograph by means of tambours. Both vagi were cut and their peripheral ends stimulated with that strength of current necessary to produce a maximal inhibitory effect. In the first experiment a 1 per cent solution of nicotine in Ringer's solution was used. In the following experiment a 0.5 per cent solution was used. These strengths of nicotine proved too great as their effect was too prolonged to permit of experimentation on successive areas. The most satisfactory strength was found to be 0.25 per cent and this strength of solution was used in all subsequent experiments. The solution was applied to the surface of the heart by means of pledgets of cotton the size of a grain of wheat, care being taken that no excess of solution was held in the cotton and that adjacent areas were protected by dry cotton. The nicotine was left in contact with the heart for a definite period of time, viz., 10 to 15 seconds. Records of the effect of stimulation of the right and left vagus were then obtained using the same strength of current that had been found necessary to produce a maximal effect. These stimulations were repeated at intervals until the effect of the nicotine had passed off. In this manner it was found possible in a number of animals to experiment upon a number of areas.

RESULTS. Table 3 shows the results of the experiments expressed

in terms of percentile loss of inhibition of both nerves following the application of nicotine to the various areas known to contain ganglia, with the exception of that of the coronary sinus area which was inaccessible to this type of experiment. The percentile loss for each nerve, as is the case in the first series of experiments, shows great variability in different animals although the average results demonstrate the same predominance of distribution of the vagi to the individual areas brought out in the experiments with the coagulating fluid. For convenience, table 4 has been prepared showing the maximal percentile loss of inhibition, hence the percentage of distribution of right and left vagi to the following areas: median side of the superior vena cava, head of the S-A node, intercaval angle, and tail of the S-A node. A comparison of the averages obtained shows that the percentage of distribution of the left vagus to the superior caval ganglia and to the ganglia of the head of the S-A node is similar, and that the percentage distribution of the right vagus is slightly less to the superior caval ganglia than to the ganglia of the head of the node. The percentage distribution of the left vagus to the intercaval ganglia is slightly greater than the percentage distribution to the ganglia of the tail of the node, while the percentage distribution of the right vagus to the intercaval ganglia and to the ganglia of the tail of the node is similar. As already intimated, the same predominance of distribution that was demonstrated by the first method employed was observed; the left nerve was predominantly distributed to the superior caval ganglia and to the ganglia of the head of the node, while the right vagus was predominantly distributed to the intercaval ganglia and to the ganglia of the tail of the node.

A comparison of the average distribution of the vagi in animals in which both nerves produce complete inhibition, with those in which the left vagus produces partial inhibition, shows in the latter a relatively poorer supply of left vagus fibers to the ganglia of the tail of the node. The left vagus predominance on the superior caval ganglia—where comparison in the same animal could be made—is likewise less than in those animals whose left vagus produced complete inhibition.

Conclusions. Both series of experiments demonstrate that there is great variability in the percentage distribution of the vagi in different animals. In some animals an equal distribution of the two nerves to any one area may be seen, or the usual relative distribution shown by the averages may be reversed. Averages, in biometrical measurements, do not, of course, represent any real objective phenomena, but

TABLE 3

Effects of nicotine on the various areas of the sino-auricular junction

DOG NUMBER	TIME OF EXPERIMENT	AREA EXPERIMENTED UPON	PERCENTAGE OF INHIBITION WITH RIGHT NERVE BEFORE NICOTINE	PERCENTAGE OF INHIBITION WITH LEFT NERVE BEFORE NICOTINE	PERCENTILE LOSS OF INHIBITION OF RIGHT NERVE AFTER NICOTINE	PERCENTILE LOSS OF INHIBITION OF LEFT NERVE AFTER NICOTINE	REMARKS
54	4:07	Superior caval ganglia	100	100	44.5	63.48	
	4:08				42.7		Complete AϕV dissociation
	4:08:30				44.8		Complete A-V dissociation
	4:11					86.7	Four periods of 2:1 rhythm
	4:12				42.9		Complete A-V dissociation
	4:17	Intercaval ganglia			80.8		Two periods of 2:1 rhythm
	4:17:30				88		One period of 2:1 rhythm
	4:18					100	
	4:21				81		Effect of nicotine passing off
	4:22:45					92	Mere slowing
	4:27:30	S-A node			88.9		
	4:28:10					81.7	Mere slowing
55	3:04:20	Intercaval ganglia	100	100			
	3:05:30				71		Regular auricular contractions; no ventricular response
	3:06:10				70.1		Regular auricular contractions; no ventricular response
	3:06:30					86	Regular auricular contractions; no ventricular response
	3:06:50					69	Regular auricular contractions; no ventricular response
	3:09	Superior caval ganglia					
	3:09:40				85.5		Regular auricular contractions; no ventricular response
	3:10:05					100	One period of 4:1 and three of 2:1 rhythm
	3:10:40					99.96	One period of 3:1 and two of 2:1 rhythm
	3:12:15				99.98		Regular auricular contractions; no ventricular response
	3:13					72.5	Effect of nicotine passing off. One period of five auricular contractions without ventricular response and six periods of 2:1 rhythm
	3:15:20	Intercaval ganglia					
	3:16:10				67.3		Regular auricular contractions; no ventricular response
	3:17:30					68.9	Regular auricular contractions; no ventricular response and four periods of 2:1 rhythm

TABLE 3—*Continued*

DOG NUMBER	TIME OF EXPERIMENT	AREA EXPERIMENTED UPON	PERCENTAGE OF INHIBITION WITH RIGHT NERVE BEFORE NICOTINE	PERCENTAGE OF INHIBITION WITH LEFT NERVE BEFORE NICOTINE	PERCENTILE LOSS OF INHIBITION OF RIGHT NERVE AFTER NICOTINE	PERCENTILE LOSS OF INHIBITION OF LEFT NERVE AFTER NICOTINE	REMARKS
55	3:19	S-A node					
	3:19:50				89.4		Regular auricular contractions; no ventricular contractions
	3:20:10					88	Regular auricular contractions; no ventricular response and incomplete heartblock
	3:21:25				79		Effect of nicotine passing off. Regular auricular contractions; no ventricular response
	3:21:45					85.5	Regular auricular contractions; no ventricular response and incomplete heartblock
56	3:51	Intercaval ganglia	100	15			Stimulation of left nerve followed by two periods of 2:1 rhythm
	3:56				75		Slowing
	3:56:45					26	Two periods of 2:1 rhythm
	3:57				65		Mere slowing
	3:57:30				80		Mere slowing
	3:58					80	Mere slowing
	4:06				0		Effect of nicotine passing off. Idioventricular beats throughout stimulation
	4:11:15					0	Effect of nicotine passed off. One period of 3:1 and five of 2:1 rhythm
	4:12	Superior caval ganglia					
	4:15:10				60		Mere slowing
	4:15:30					99.9	
	4:17:10				59.6		Mere slowing
	4:18:50					0	Effect of nicotine passed off. 2:1 rhythm throughout stimulation
	4:19:30				0		Effect of nicotine passed off
	4:25	Intercaval ganglia					
	4:27				75.5		Mere slowing
	4:27:15					99.6	2:1 rhythm throughout stimulation
	4:27:40				99.9		Slowing
	4:28:30					99.5	Slowing
	4:34				40		Effect of nicotine passing off. Marked slowing and sinus arrhythmia
	4:34:50					0	Effect of nicotine passed off. Six periods of 2:1 rhythm

TABLE 3—*Continued*

DOG NUMBER	TIME OF EXPERIMENT	AREA EXPERIMENTED UPON	PERCENTAGE OF INHIBITION WITH RIGHT NERVE BEFORE NICOTINE	PERCENTAGE OF INHIBITION WITH LEFT NERVE BEFORE NICOTINE	PERCENTILE LOSS OF INHIBITION OF RIGHT NERVE AFTER NICOTINE	PERCENTILE LOSS OF INHIBITION OF LEFT NERVE AFTER NICOTINE	REMARKS
57	2:52:20	Intercaval ganglia	100	60			A-V rhythm during left vagus stimulation
	3:00:10				71.4		One period of 3:1 and three of of 2:1 rhythm
	3:00:30					33.3	Two periods of 2:1 rhythm
	3:08:05				0	0	Effect of nicotine passed off
	3:09	Superior caval ganglia					
	3:10:10				63.5		One period of 3:1 and three of 2:1 rhythm
	3:10:45					100	
	3:15				0	0	Effect of nicotine passed off. Right vagus gives complete inhibition. Left vagus inhibits auricles only, ventricles beating slowly
	3:16	Head of S-A node					
	3:17				27.5		Idioventricular beats throughout stimulation
	3:17:20					69.5	Five periods of 2:1 rhythm
	3:18					53.4	One period of 3:1 and four of 2:1 rhythm
	3:19:10					54.7	One period of 3:1 and three of 2:1 rhythm
	3:19:30				0		The tracing indicates a shifting of the site of impulse formation to the A-V node. The S-A node was, therefore, depressed by stimulation of right vagus: the effect of nicotine on the S-A node must have passed off.
	3:26:45	Tail of S-A node					
	3:27:50				77.4		Regular auricular contractions; no ventricular response
	3:28:50					59	Five periods of 2:1 rhythm
58	3:37:20	Superior caval ganglia	100	100			
	3:41				0		Complete inhibition
	3:41:20					34.7	Showing of auricles; no ventricular response
	3:41:40					62.8	One period of 4:1 and one of 2:1 rhythm

TABLE 3—*Continued*

DOG NUMBER	TIME OF EXPERIMENT	AREA EXPERIMENTED UPON	PERCENTAGE OF INHIBITION WITH RIGHT NERVE BEFORE NICOTINE	PERCENTAGE OF INHIBITION WITH LEFT NERVE BEFORE NICOTINE	PERCENTILE LOSS OF INHIBITION OF RIGHT NERVE AFTER NICOTINE	PERCENTILE LOSS OF INHIBITION OF LEFT NERVE AFTER NICOTINE	REMARKS
58	3:42:30				0		
	3:43					53 5	Sinus arrhythmia and one period of 2:1 rhythm
	3:48:50	Intercaval ganglia					
	3:51:25				72 3		Five periods of 2:1 rhythm
	3:51:45					83 4	Regular auricular contractions; no ventricular response
	3:53:35				63 4		Effect of nicotine passing off. One period of 3:1 and two of 2:1 rhythm
	4:00					35	Effect of nicotine passing off. One period of 2:1 rhythm. Slowing of entire heart
59	2:59:30	Head of S-A node	100	18 6			One period of 3:1 and four of 2:1 rhythm on stimulation of left vagus
	3:05:30				70 7		Seven auricular beats; no ventricular response
	3:05:50				70		Five auricular beats without ventricular response then three periods of 2:1 rhythm
	3:06:10					75	Mere slowing
	3:25					84 1	Mere slowing
	3:37:45				0		Idioventricular beats (96 per min.) throughout time of stimulation
	3:38:20					87 36	Mere slowing
	3:40:45	Tail of S-A node					
	3:42:40				83 4		Mere slowing
	3:43					87 64	Mere slowing
	3:45:30				100		
	3:48					53 7	Slowing
	3:58:15				0		
60	3:55:30	Tail of S-A node	100	100			
	4:00:30				65 5		Mere slowing
	4:01					78 5	Mere slowing
	4:03:30				59 3		Slowing
	4:03:55					81 5	Slowing
	4:08:55				0		Effect of nicotine passed off
	4:09:15					57 1	
	4:12					53 4	
	4:13:10	Intercaval ganglia					

TABLE 3—*Continued*

DOG NUMBER	TIME OF EXPERIMENT	AREA EXPERIMENTED UPON	PERCENTAGE OF INHIBITION WITH RIGHT NERVE BEFORE NICOTINE	PERCENTAGE OF INHIBITION WITH LEFT NERVE BEFORE NICOTINE	PERCENTILE LOSS OF INHIBITION OF RIGHT NERVE AFTER NICOTINE	PERCENTILE LOSS OF INHIBITION OF LEFT NERVE AFTER NICOTINE	REMARKS
60	4:15:50					81.7	Mere slowing
	4:16:20				69.5		Mere slowing
	4:16:50					89.2	Mere slowing
	4:35:40					82.5	
	4:39:45				0		Effect of nicotine off
	4:09:15					57.1	
	4:12					53.4	
	4:13:10	Intercaval ganglia					
	4:15:30					81.7	Mere slowing
	4:16:20				69.5		Mere slowing
	4:16:50					89.2	Mere slowing
	4:35:40					82.5	
	4:39:45				0		Effect of nicotine off
	4:40	Head of S-A node					
	4:40:15					96	
	4:40:50				54		Slowing
	4:43:50					66	Slowing
	4:44:10				41.3		Slowing
	4:50:10				0		Effect of nicotine off
	4:51					56	Slowing
	4:54	Superior caval ganglia					
	4:54:30				91.5		Slowing
	4:55					87.5	Slowing
	4:55:25				39.5		Slowing
	4:55:50					87.5	Slowing
	5:04:05				39.4		Marked slowing with sinus arrhythmia
	5:04:25					62.5	Slowing
61	3:11:10	Head of S-A node	100	63.8			Irregular auricular beats; no ventricular response
	3:20				100		
	3:20:20					90	Complete A-V block
	3:21:10				82.7		Stronger stimulation. Complete A-V block
	3:21:40					100	Seven periods of complete A-V block followed by incomplete
	3:33				0		Effect of nicotine off
	3:33:50					60.1	Complete A-V block
	3:38:10	Superior caval ganglia					
	3:39:20					98	
	3:40:25				84.6		Three periods of 2:1 rhythm
	3:40:50					87.4	
	3:50:20				77.9		
	3:50:40				0		One period of 2:1 rhythm
	3:50:55					86.67	Stronger stimulation. Idioventricular beats; one retrograde (V-A) auricular beat

TABLE 3—*Continued*

DOG NUMBER	TIME OF EXPERIMENT	AREA EXPERIMENTED UPON	PERCENTAGE OF INHIBITION WITH RIGHT NERVE BEFORE NICOTINE	PERCENTAGE OF INHIBITION WITH LEFT NERVE BEFORE NICOTINE	PERCENTILE LOSS OF INHIBITION OF RIGHT NERVE AFTER NICOTINE	PERCENTILE LOSS OF INHIBITION OF LEFT NERVE AFTER NICOTINE	REMARKS
61	4:21:50				0		Stronger stimulation. Mere slowing
	4:22:15					92.79	Stronger stimulation. Idioventricular beats; one retrograde (V-A) auricular beat
	4:24	Intercaval ganglia					Stronger stimulation. Mere slowing
	4:26:20				100		
	4:27:15					83.7	Two periods of 2:1 rhythm
62	3:00	Superior caval ganglia	100	100			
	3:03:35				0		Idioventricular beats during stimulation
	3:03:55					85	Idioventricular beats during stimulation
	3:04:10					83.3	One period of 3:1 and two of 2:1 rhythm
	3:04:20				48.1		One period of 3:1 and two of 2:1 rhythm
	3:07:30				17		Sinus arrhythmia
	3:08					60	Idioventricular beats during stimulation
	3:12:30				0		One retrograde (V-A) contraction
	3:12:50					31	Idioventricular beats during stimulation
	3:14:20	Head of S-A node					Two idioventricular beats
	3:15:10				66.6		
	3:15:30					72	Two periods of 2:1 rhythm
	3:27:10				36		Two periods of 2:1 rhythm
	3:27:30					82	One idioventricular beat
	3:30:15				36		
	3:30:55					66.7	One idioventricular beat
	3:43:20				0		
	3:45:25					43.6	One idioventricular beat
	3:48:20	Intercaval ganglia					
	3:49:20				77.6		Two periods of 2:1 rhythm
	3:49:40					77.6	One period of 2:1 rhythm
	3:50:40				74.8		
	3:51					75	
	4:14:05				40		One idioventricular beat and one period of 2:1 rhythm
	4:14:30					63.6	
63	2:50:30		100				
	2:50:50			24.6			No A-V block
	2:52:50	Intercaval ganglia					

TABLE 3—*Concluded*

DOG NUMBER	TIME OF EXPERIMENT	AREA EXPERIMENTED UPON	PERCENTAGE OF INHIBITION WITH RIGHT NERVE BEFORE NICOTINE	PERCENTAGE OF INHIBITION WITH LEFT NERVE BEFORE NICOTINE	PERCENTILE LOSS OF INHIBITION OF RIGHT NERVE AFTER NICOTINE	PERCENTILE LOSS OF INHIBITION OF LEFT NERVE AFTER NICOTINE	REMARKS
63	2:53:45				76.6		
	2:54:10					20.7	
	3:00:40				50.5		
	3:01:05					18.3	
64	3:07:50		100				One idioventricular beat
	3:08:10			100			Heart escapes from inhibition more readily than with right vagus. One idioventricular beat
	3:10:50	Head of S-A node					
	3:11:40				52.8		Two periods of 2:1 rhythm
	3:12:50					55.5	Three periods of 2:1 rhythm
	3:14:30					64	Two periods of 2:1 rhythm
	3:16:40				48.7		One period of 2:1 rhythm
	3:17					0	
	3:17:20				0		
	3:30:15	Intercaval ganglia					
	3:31:05				58.6		Two periods of 2:1 rhythm
	3:32:20					58.3	One period of 2:1 rhythm
	3:34				40.7		One period of 2:1 rhythm
	3:34:30					42.7	
	3:38					0	
	3:38:15				0		
	3:40:15	Tail of S-A node					
	3:40:55				45.3		Three periods of 2:1 rhythm
	3:41:15					53.5	One period of 3:1 and two of 2:1 rhythm
	3:41:45				64.3		One period of 3:1 and two of 2:1 rhythm
	3:41:55					60.5	One period of 3:1 and two of 2:1 rhythm
	3:46:05				45.9		Temporary complete inhibition; then two periods of 2:1 rhythm
	3:46:25					0	
	3:47:35				0		
	3:54:40	Intercaval ganglia					
	3:55:10				68.2		One period of 2:1 rhythm
	3:55:20					48.7	Two periods of 2:1 rhythm
	3:56				49.8		
	3:56:30					42.1	Temporary complete inhibition
	3:56:50				0		
	3:57:20					0	

serve only as an index of the expected frequency with which the phenomena may occur. Hence it is necessary in all such investigations to use as large a number of animals as possible.

Attention has already been called to the marked similarity between the average percentile distribution, on the one hand, of the left vagus to the superior caval ganglia and to those of the head of the node and, on the other hand, of the right vagus to the intercaval ganglia and to

TABLE 4

Percentile loss of chronotropic influence of right and left vagus in relation to the various parts of the sino-auricular junction following the application of nicotine

DOG NUMBER	SUPERIOR CAVAL GANGLIA		HEAD OF S-A NODE		INTERCAVAL GANGLIA		TAIL OF S-A NODE	
	Right vagus	Left vagus	Right vagus	Left vagus	Right vagus	Left vagus	Right vagus	Left vagus
54	44.8	86.7						
55					71.0	86.0		
56	60.0	99.9			80.0	80.0		
56					99.9	99.5		
57	63.5	100.0	27.5	69.5	71.4	33.3	77.4	59.0
58	0	62.8			72.3			
59			70.7	87.4			100.0	0
60	91.5	+*	54.0	+*	69.5	+*	65.5	81.5
61	84.6		100.0	100.0	100.0	0		
62	48.1	85.0	66.6	+*	77.6	+*		
63					76.6	84.4		
64			52.8	55.5	58.6	58.3	64.3	60.5
Averages....	56.07	86.88	61.93	78.1	77.69	63.07	76.8	50.2

* The plus sign indicates that there was a further loss of inhibition when nicotine was applied to this area but, owing to the circumstance that the effect of nicotine previously applied had not entirely passed off, the additional loss could not be accurately estimated.

those of the tail of the node. That this similarity is not due to any chance spreading of the nicotine to the contiguous areas is well shown by the results obtained in individual experiments. (See exper. 57 and 62, table 4.)

The striking variability in the percentile distribution of the vagus fibers may be due to the chance distribution of sympathetic cells at the time of their migration. The observations are obviously too few, however, to determine whether or not this distribution follows the

curve of probability. This variability may be due also to the shifting of the ganglion cells and their related vagus fibers during the development of the heart. In human embryos the sympathetic nerve cells have reached the heart before the end of the sixth week (41). The time of migration of the sympathetic cells coincides with the period of rapid increase in the size of the atria and of the absorption of the right horn of the sinus venosus into the wall of the right atrium.

A careful examination of the results obtained by Garrey (7) and by Cruickshank (8) in the turtle shows that in this animal the variability of distribution of the vagi is very much less extensive. The disappearance of the sinus venosus as a separate chamber—which may be regarded as a loss of specialization—is, therefore, accompanied by an increase in the variability of distribution of the vagus fibers to the sino-auricular junction.

SUMMARY

Ganglia in relation with the vagus nerves are found throughout the sino-auricular junction of the dog's heart. These ganglia occur in five groups: *1*, A superior caval group, on the median side of the superior vena cava; *2*, a group adjacent to, or coterminous with the head of the sino-auricular node; *3*, a group, similarly situated, in reference to the tail of the S-A node; *4*, an intercaval group penetrating a certain distance into the tubercle of Lower; *5*, a coronary sinus group, in the tissue between the mouth of the coronary sinus and the limbus of the fossa ovalis.

These various groups of ganglia are related to both vagi. Average computations, based on the effects of destruction of these areas by a coagulating fluid and on the effects of the application of nicotine, show that the left vagus is predominantly distributed to the superior caval ganglia; that the same relationship obtains for the ganglia of the head of the S-A node; that the intercaval ganglia are predominantly supplied by the right vagus; that the same right vagal predominance holds true for the ganglia of the tail of the S-A node; and, finally, that the left vagus predominates in its distribution to the coronary sinus ganglia, this nerve being, in the majority of instances, the only one to be distributed to these ganglia.

The primary destruction of the area of the coronary sinus containing the ganglia is not followed by any disturbance of atrio-ventricular conduction. It would seem that the inhibitory effect of the left vagus may be in part exercised on the sinus tissue of this area. In any event,

there is no valid reason, either phylogenetically or functionally, for assigning this tissue to the atrio-ventricular node.

The customary greater inhibitory power of the right vagus is directly related to its more extensive distribution to the ganglia of the sino-auricular node. In those instances in which the left vagus fails to produce complete inhibition its percentile distribution to the ganglia of the S-A node and to the superior caval ganglia is less than in those instances in which it produces complete inhibition.

Great variability is exhibited in the percentile distribution of both vagus nerves in different animals, the average order of predominance being sometimes reversed in individual cases.

In conclusion, I take pleasure in expressing my thanks to Dr. R. H. Baldwin and Mr. W. W. Daniel for their unstinted help in the course of the experiments and in the preparation of the histological material.

BIBLIOGRAPHY

(1) Weber: Handwörterb. d. Physiol. (Wagner), 1846, 42–47. (Quoted from Bayliss: Principles of general physiology, 1915, 682.)
(2) Meyer: Das Hemmungsnervensystem des Herzens, Berlin, 1869.
(3) Gaskell: Journ. Physiol., 1883, iv, 82.
(4) Mills: Journ. Physiol., 1885, vi, 247.
(5) Kazem-Beck: Arch. f. Anat. u. Physiol., 1888, 325. (Quoted from Garrey, q. v. infra.)
(6) Guyénot: Compt. rend. Soc. d. Biol., 1907, lxii, 1032.
(7) Garrey: This Journal, 1911, xxviii, 330.
(8) Cruickshank: This Journal, 1920, liv, 217.
(9) Gaskell: Journ. Physiol., 1887, viii, 404.
(10) Wybauw: Arch. intern. d. Physiol., 1910, x, 78.
(11) Lewis, Oppenheimer and Oppenheimer: Heart, 1910–11, ii, 147.
(12) Rothberger and Winterberg: Arch. f. d. gesammt. Physiol., 1910, cxxxv, 559.
(13) Flack: Journ. Physiol., 1910–11, xli, 64.
(14) Marchand and Meyer: Arch. f. d. gesammt. Physiol., 1912, cxlv, 401.
(15) Cohn: Journ. Exper. Med., 1912, xvi, 732.
(16) Ganter and Zahn: Arch. f. d. gesammt. Physiol., 1912, cxlv, 335.
(17) Robinson and Draper: Journ. Exper. Med., 1911. xiv, 217; 1912, xv, 14.
(18) Lewis: Heart, 1914, v, 247.
(19) Lewis, Meakins and White: Phil. Trans. Royal Society, London, 1914, B, ccv, 375.
(20) Schlomovitz, Eyster and Meek: This Journal, 1915, xxxvii, 177.
(21) Keith and Flack: Journ. Anat. and Physiol., 1907, xli, 172.
(22) Aschoff: Zentralbl. f. allg. Path. u. path. Anat., 1910, xxi, 433.
(23) Vignal: Ecole prat. d. hautes Etudes, Trav., Paris, 1881, vi, 186.
(24) Remak: Arch. f. Anat. u. Physiol., 1844, 463.

(25) SCHKLAREWSKI: Nachricht. v. d. K. Gesellsch. d. Wissensch. u. d. Georg-August Univ., Goethingen, 1872, 426.
(26) DOGIEL: Arch. f. mikr. Anat., xiv, 470.
(27) OPPENHEIMER AND OPPENHEIMER: Journ. Exper. Med., 1912, xvi, 613.
(28) KOCH: Med. Klinik, 1912, no. 3.
(29) MEIKLEJOHN: Journ. Anat. and Physiol., 1913–14, xlviii, 1, 378.
(30) LISSAUER: Arch. f. mikr. Anat., 1909, lxxiv, 217.
(31) SCHWARTZ: Arch. f. mikr. Anat., 1899, liii.
(32) WALEDINSKY: Anat. Anz., 1910, xxxvii, 465.
(33) MORRISON: Journ. Anat. and Physiol., 1911–12, xlvi, 319.
(34) WILSON: Proc. Roy. Soc., 1909, B, lxxxi, 151.
(35) ARGAUD: Arch. d. Mal. d. Coeur, etc., 1911, iv, 638.
(36) WIGGERS: This Journal, 1916, xl, 218.
(37) MEEK AND EYSTER: This Journal, 1914, xxxiv, 368.
(38) ZAHN: Arch. f. d. gesammt. Physiol., 1913, cli, 247.
(39) ERLANGER AND BLACKMAN: This Journal, 1907, xix, 125.
(40) LANGLEY AND DICKINSON: Proc. Roy. Soc., 1889, xlvi, 423.
(41) STREETER: in KEIBEL AND MALL'S Manual of human embryology, Philadelphia, 1912, ii, 144.

STUDIES IN FATIGUE

XIII. The Staircase Phenomenon in Mammalian Skeletal Muscle

CHARLES M. GRUBER

From the Department of Pharmacology, Washington University School of Medicine, St. Louis

Received for publication October 21, 1922

The production of "treppe" and its cause have been studied by many experimenters (1). Lee (2), in 1907, working upon both warm- and cold-blooded animals, came to the conclusion that it was due to the physiological action of each of the commonly recognized fatigue substances: carbon dioxide, paralactic acid and monopotassium phosphate. These substances act in two opposite modes, he writes, the appearance of the one or the other mode being dependent upon the quantity of the substance which is present. In moderate quantity, or smaller quantity for a longer time, each substance is depressing or fatiguing. If present in small quantity or a moderate quantity for a brief time, it causes an augmentation of activity, which is characterized by an increase in irritability and working power, an increase in the height to which the load is lifted and an increase in the total amount of work performed. These conclusions are not supported by Fröhlich (3) nor by Adrian (4).

Fröhlich working upon curarized and non-curarized frog's skeletal muscles found that the increase in the height of contraction "treppe" was accompanied by an increase in the duration of the contraction phase. The increase in the time of the contraction phase may lead to an actual increase in the tension exerted by the whole muscle, he believes, because the longer the duration of the contraction phase the more chance is there that all parts of the muscle will be fully contracted at the same moment (5). He believes also that the same conditions exist in the nerve-muscle preparation as in the muscle upon repeated stimulation (6).

Adrian (4) experimented with the sartorius muscle of the frog which he stimulated with break induction shocks every one to three seconds

through its nerve free end (pelvic end). The muscles were perfused with fluids varying from pH 10 to pH 4. When the contractions were recorded by "a very light tension lever with small moment of inertia" the staircase effect was never observed, but it did appear when the tension lever was discarded and an isotonic lever was used in its place. Its appearance bore no relation to the pH. This observer explains the cause of "treppe" upon the bases of Fröhlich's work, i.e., increased duration of the contraction phase in the early stages of fatigue, and of his own, on the inertia of the writing lever.

Inasmuch as the author had not recalled an experiment in which the staircase phenomenon had been absent in fatigued intact mammalian skeletal muscles contracting isometrically, this work was undertaken to determine if the phenomenon is dependent upon the increased duration of the contraction phase in warm blooded animals as is believed to be the case in cold blooded animals.

METHOD. Usually cats anesthetized with ether were used. In those cases in which curare was employed, urethane (2 grams per kilo, body weight by stomach) was used as the anesthetic. By making a small slit through the skin on the outer side of the thigh, the anterior tibial nerve was isolated, cut, and its distal end fastened in a Sherrington shielded electrode (7). The electrode was held in place by fastening around it, with paper clips, the two flaps of skin.

Through another slit in the skin on the same limb the tendon of the tibialis anticus muscle was isolated from its insertion. The tendon was then fastened to a muscle lever by a string passing about two delicate pulleys. These pulleys were arranged so that the muscle pulled in its normal direction. One cotton cord looped about the hock and another around the foot just below the original insertion of the tendon bound the leg to the animal board. This nerve muscle preparation had its normal blood supply, unaltered except by the cutting of the anterior tibial nerve.

The stimulating current was a maximum break induction shock. A revolving drum, upon which the record was written, was propelled at a uniform rate by a spinning device (8). That the rotation of the drum was uniform in rate can be seen in figure 1. The drum was spun five times and tuning fork records taken. This drum automatically broke the platinum contact key which was in the primary circuit of the stimulating current, in its revolution. The muscle was excited at the rate of 100 times a minute. The 1st, 10th, 20th, 100th and 200th contractions were recorded. The fiber and brass wheel which made

and broke the primary circuit was 6 inches in diameter and was rotated at a uniform speed by an electrically driven motor. The secondary poles of the inductorium were connected to the shielded electrodes on the anterior tibial nerve. In those instances in which the muscle was curarized the curare was injected into the external jugular vein in a 1.5 per cent solution and the muscle stimulated electrically through needle platinum electrodes thrust directly into the muscle. The records were taken after the muscle failed to respond to indirect excitation. Artificial respiration was induced as soon as the animal ceased to breathe.

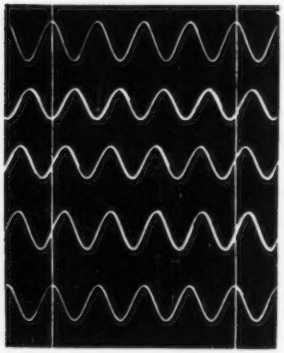


Fig. 1. Tuning fork records showing the uniformity with which the drum rotated in five consecutive records.

The muscle lever consisted of a piece of light bamboo fastened to a metal axis on one end and tipped with a small parchment paper writing point on the other. The entire lever weighed 2.5 grams, but that part from the attachment of the tendon and spring which was 3.5 cm. from the axis to the tip of the writing point weighed only 0.6 gram. The spring in most instances had an initial tension of 70 grams the moment the muscle began to contract, which increased 7 grams for each centimeter excursion of the muscle lever on the drum surface. The lever magnifies the contraction on the record three times. An electrically vibrated tuning fork marking 1/100 seconds was used in recording the time interval.

Results. My results upon the intact cat's skeletal nerve-muscle do not confirm those of Fröhlich (3) upon the frog's skeletal muscle. Forty-five readings were made upon 31 animals. (See table 1.) In the readings made of the 10th or 20th contractions the average shortening of the contraction phase was 30 per cent, of the contraction and relaxation phases combined, 17 per cent; but the average height of the contraction remained the same.

At the end of the 100th contraction in the forty-five readings the average durations of the contraction phase and the contraction and relaxation phases combined were decreased by 22 and 20 per cent respectively. In five experiments the duration of the contraction phase remained unaltered. The height of the contractions increased 57 per cent.

TABLE 1

A summary of the results on 31 cats under ether anesthesia. Anterior tibial muscle stimulated indirectly through its nerve and contracting against a spring having an initial tension of 70 grams which increased 7 grams for each centimeter on the drum's surface. The duration of the contraction phase (I) and the contraction and relaxation phases (II) are recorded in 1/100 seconds. The height of the contraction (III) is in millimeters

FIRST CONTRACTION			10TH OR 20TH CONTRACTION			100TH CONTRACTION			200TH CONTRACTION		
I	II	III	I	II	III	I	II	III	I	II	III
2.7	7.5	9	1.5	5.4	9	1.8	4.7	18	1.9	5.0	22
2.8	7.7	9	1.7	5.5	8	1.4	4.8	12	1.7	4.9	15
2.8	10.0	10	1.6	7.0	12	1.8	6.2	17	1.5	7.1	20
3.0	8.7	15	1.8	7.0	15	2.1	7.1	20	2.3	8.0	24
2.6	7.0	12	2.4	6.2	10	1.7	6.5	14	2.0	6.5	16
2.9	6.3	12	1.9	4.7	11	2.0	4.7	17	2.0	5.0	21
2.9	7.8	18	1.4	6.8	18	2.9	6.3	27	2.9	5.8	30
2.4	6.6	13	1.4	5.1	9	2.4	5.1	14	2.4	5.3	15
3.5	8.7	20	3.0	7.3	26	3.0	7.0	30	3.1	7.0	34
2.0	12.0	12	2.0	9.0	9	2.3	13.3	23	2.5	13.3	27
2.9	5.7	11	1.6	4.2	11	1.4	4.6	20	1.4	4.6	21
1.7	5.0	11	1.5	4.3	13	1.2	4.0	19	1.4	4.0	18
1.4	6.0	12	1.5	4.0	6	1.4	4.1	13	1.3	4.1	15
1.8	5.5	15	1.5	5.0	16	1.3	4.1	24	1.4	4.2	25
2.9	7.7	10	1.7	5.4	9	1.9	4.6	13	1.9	4.6	15
2.7	7.9	9	1.5	5.7	9	2.0	4.9	18	2.1	5.1	22
2.8	10.4	11	1.9	7.0	13	2.0	6.2	17	2.0	7.2	21
2.9	8.5	15	2.1	7.0	15	2.1	7.3	21	2.2	8.0	24
2.7	7.0	14	2.3	6.2	11	2.2	7.0	17	2.1	7.0	19
2.7	7.8	10	1.8	5.5	10	2.0	4.7	16	2.0	5.0	19
2.5	4.7	9	1.5	3.9	11	1.6	4.0	18	2.3	4.7	22
2.1	5.0	8	1.5	4.0	9	1.7	4.5	16	1.7	5.7	19
2.4	5.3	8	2.0	4.5	10	2.0	4.7	15	2.4	4.6	20
2.9	5.7	13	2.0	5.0	14	1.7	4.7	22	2.3	5.1	25
2.5	4.9	9	1.5	4.2	9	1.6	4.2	17	2.5	4.7	21
2.4	5.0	14	1.7	4.3	17	2.0	4.3	23	2.0	4.8	24
2.5	5.0	8	2.0	4.0	10	2.0	5.0	15	2.3	4.5	20
2.8	5.9	17	2.6	5.6	23	2.6	5.6	31	2.8	5.8	31
3.2	6.7	16	1.5	6.0	20	2.3	5.6	31	2.5	5.7	35
3.2	8.0	15	1.6	6.7	19	2.4	5.2	28	2.5	5.7	34
4.4	8.0	23	3.7	6.7	32	3.1	6.9	38	3.1	6.7	40
3.0	6.2	20				2.1	5.1	39	2.1	5.7	38
2.4	5.2	17				2.1	5.1	28	2.1	5.1	28
2.4	6.0	14				1.7	5.1	16	1.7	5.0	15
5.3	13.0	16				3.5	11.0	33	3.6	13.5	37
3.7	8.9	19				2.8	6.9	27	2.8	7.1	31
1.5	4.2	8				1.5	4.0	17	1.4	4.2	18
4.0	10.8	13				2.8	7.2	21	2.4	7.3	27

TABLE 1—*Concluded*

	FIRST CONTRACTION			10TH OR 20TH CONTRACTION			100TH CONTRACTION			200TH CONTRACTION		
	I	II	III	I	II	III	I	II	III	I	II	III
	2.3	11.5	18				2.3	9.2	26	2.4	9.2	28
	2.5	7.4	16				2.0	7.2	28	2.5	8.2	35
	3.9	8.8	19				2.9	6.8	27	2.7	6.7	30
	2.4	10.3	14				1.7	9.4	22	2.1	9.1	18
	1.5	4.5	10				1.3	4.0	21	1.2	4.6	23
	2.5	6.8	15				1.6	5.1	17	1.8	5.2	26
	3.0	9.9	22				2.7	7.5	32	2.5	8.1	36
Average for first 31 readings..	2.7	7.0	13	1.9	5.6	13	2.0	5.5	20	2.1	5.8	23
Total average.....	2.7	7.4	14	1.9	5.6	13	2.1	5.7	22	2.2	6.0	25

The 200th contraction showed an average decrease of 19 per cent in the duration of the contraction phase and a decrease of 19 per cent in the duration of the contraction and relaxation phases when compared with those of the 1st contraction. In six experiments the duration of the contraction phase remained unchanged and in one instance only was it lengthened beyond that of the 1st contraction. The height of the contractions was, however, increased by 79 per cent.

In these experimets the average duration of the latent period for the 31 experiments was 0.017 second for the 1st contraction and 0.015 second for the 100th contraction.

Figure 2 is a record showing the 1st, *1*, 10th, *2*, 100th, *3*, and the 200th, *4*, contractions. If we compare in this figure curve *1* with curves *2*, *3* and *4*, we at once note that the duration of the contraction phases is shorter and the height of the contractions much increased in the three latter curves.

Measurement reveals the fact that the time of the contraction phase is decreased by 15 per cent in *2*, and by 32 per cent in *3* and *4;* nevertheless the height of the contractions is increased by 39, 65 and 73 per cent respectively.

Figure 3 is a similar curve except that the duration of the contraction phase in both the 100th and the 200th contractions is longer than that seen in the 10th contraction. Curve *1* is the 1st, *2* the 10th, *3* the 100th and *4* the 200th contraction. Upon measuring the time

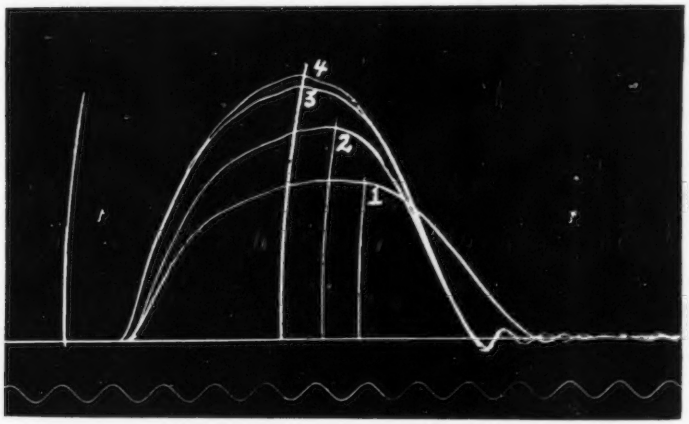


Fig. 2. A record taken from the anterior tibial muscle contracting against a spring having an initial tension of 70 grams. Increased tension per centimeter on drum, 7 grams. Muscles stimulated through anterior tibial nerve (peroneus communis). *1*, 1st; *2*, 10th; *3*, 100th, and *4*, 200th contractions. In this and all following figures, unless otherwise stated, the time interval is in 1/100 seconds. All records read from left to right.

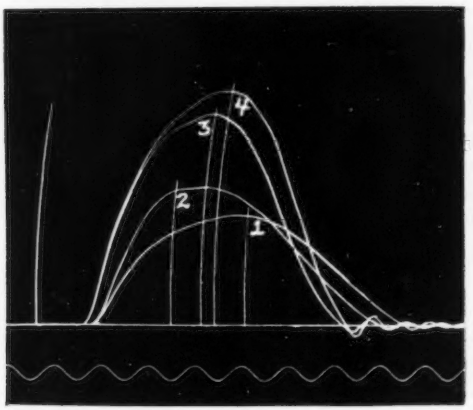


Fig. 3. Record same as that in figure 2, but another animal.

of the contraction phases and the height of the contractions in *2*, *3* and *4* and comparing them with curve *1*, there is observed the same phenomenon as in figure 2. The time of the contraction phases was decreased 50, 27 and 15 per cent respectively but the height of the contractions in curves *2*, *3* and *4* was increased by 25, 94 and 119 per cent respectively.

Curarized muscle. The results upon eight cats anesthetized with urethane, later curarized, confirm the findings on the nerve-muscle preparations. In a few instances the contraction time was the same for the 1st and the subsequent contractions, nevertheless the height of the contractions increased. In others the duration of the contraction phases of the subsequent contractions shortened but the height of the contraction was increased. Figures 4 and 5 are two such records. In both instances the initial tension was 35 grams which was increased by 7 grams for each centimeter the writing lever wrote perpendicularly on the drum's surface. In figure 4 the duration of the contraction phase for the 1st contraction, *1*, was 0.024 second, which was decreased in the subsequent contractions; 20th *2*, 100th, *3*, and 200th, *4*, to about 0.021 second, or a decrease of about 12 per cent. However, the height of the contractions was increased by 80, 180 and 220 per cent respectively.

In figure 5 the maximum shortening of the 1st contraction appears to be at *1*, but upon more careful examination it is found to be at *1'*, making its duration 0.043 second. The duration of the contraction phases of the 20th, *2*, 100th, *3*, and 200th, *4*, contractions respectively was decreased 42, 38 and 31 per cent. The height of these contractions was increased by 25, 100 and 137 per cent respectively.

Does activity increase the irritability of the muscle? The change in irritability during "treppe" can readily be demonstrated in the muscle by determining the threshold stimulus before and at the height of the staircase phenomenon. To secure the slightest contraction before and at the height of "treppe" during these experiments in which it was tested, the author frequently observed that the secondary coil could be moved 0.5 to 2 cm. further away from the primary coil at the height of "treppe."

In many instances in which the make shock was minimal in the beginning it became gradually submaximal during the staircase phenomenon and the secondary coil had to be removed some centimeters before the make shock contraction disappeared. These results confirm the early findings of Lee (2).

What effect has asphyxia upon the height of contraction? Adrian (4) was unable to obtain the staircase effect by perfusing a frog's muscle with fluids varying from pH 10 to pH 4. He observed that the contractions diminish in height to complete exhaustion without "treppe" although the muscle had been perfused for 4 hours. Lee (2) showed that if small quantities of monopotassium phosphate, paralactic acid

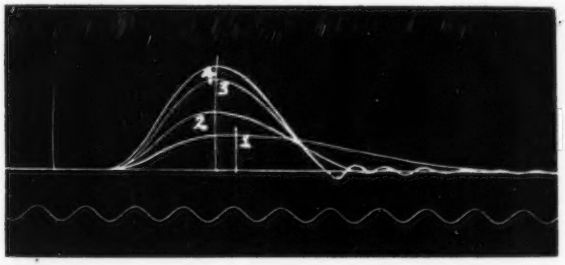


Fig. 4. A record taken from the curarized anterior tibial muscle contracting against a spring having an initial tension of 35 grams with 7 grams added for each centimeter on the vertical drum surface. Muscle stimulated directly through platinum needle electrodes thrust into the muscle. *1*, 1st; *2*, 20th; *3*, 100th, and *4*, 200th contractions.

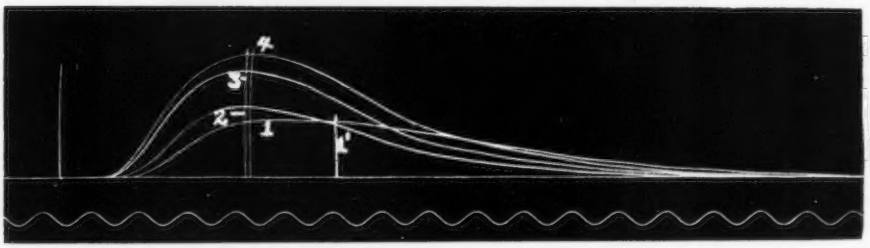


Fig. 5. Conditions the same as in figure 4 but from another animal.

and carbon dioxide were injected into the circulation an increase in the height of muscular contraction similar to the "treppe" phenomenon resulted. He found large amounts for a brief period, or small amounts for a longer period to have the opposite action. These results have been confirmed by the author (unpublished results). The author noted that the effect of asphyxia alone was not uniform for the muscles of the different animals. Asphyxia alone produced in some instances an

increase in the height of the contractions with or without a decrease in the time of the contraction phase; in others a decrease only in the height of the contraction was observed. If, however, the muscle was stimulated rhythmically only an increase in the height of the contraction was observed, which was usually accompanied by a decrease in the duration of the contraction phase though in a few cases it was increased. Figure 6 is a curve showing the effect of asphyxia alone and with exercise upon the same muscle. The animal was prepared in the usual

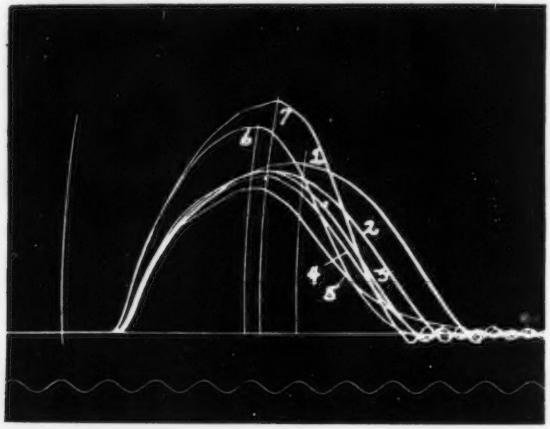


Fig. 6. A record taken from the anterior tibial muscle of the cat. Animal killed after curve *1* was written. Curves *2*, *3*, *4* and *5* were recorded 2, 4, 6 and 12 minutes respectively after the animal was dead. The muscle was excited 100 times a minute after curve *5* was written and the 10th, *6*, and the 100th, *7*, contractions recorded.

manner after which curve *1* was recorded. The abdominal aorta was then quickly ligated so that the minimum amount of ether could reach the muscle when the animal was killed with it. Curves *2*, *3*, *4* and *5* were recorded 2, 4, 6 and 12 minutes respectively after the animal was dead. The muscle was then excited electrically and the 10th contraction, *6*, and the 100th contraction, *7*, written on the drum. Upon comparing curves *2*, *3*, *4* and *5* with curve *1*, we find that as the time of the contraction phase diminished, the height of the contractions decreased, which is in harmony with Fröhlich's theory (3). If, however, we compare curves *6* and *7* with curve *1*, we see at once the reverse

condition, i.e., a decreased time of contraction phase with a marked increase in the height of the contraction, which is contrary to expectation. It seems, therefore, from these results that activity increases the irritability more rapidly than asphyxia alone.

Discussion. From these results it is evident that the contraction phase in cats' intact skeletal nerve-muscle and curarized muscle preparations, does not increase during "treppe" when compared with the duration of the phase of contraction of the first response. Fröhlich's theory (3) and the conclusions drawn from his results upon the skeletal nerve-muscle and curarized muscle preparations of frogs, that the "treppe" phenomenon is due to a lengthening of the contraction phase thus permitting a larger part of the muscle to contract simultaneously, is not supported by these findings.

Lee in 1898 (9) pointed out that all previous knowledge of fatigue, limited largely to the frog, is incomplete. He showed that the essential element in the course of fatigue common to all three experimental animals—frog, turtle and cat—is a decrease of the lifting power. He noted that the slowing of the phase of contraction, while slight in the frog, is great in the turtle and apparently wanting altogether in the white muscle of the cat. This difference in cold and warm blooded muscles was also shown to exist by Rollett (10). The increased time of the contraction phase found in frogs' skeletal muscle upon which Fröhlich bases his theory as to the cause of "treppe" plays no part in the actual production of "treppe" in cat's skeletal muscle, since it is wanting there. The author has been able to show repeatedly that the threshold stimulus is less when the muscle is at the height of "treppe" than at the beginning of the fatigue.

Adrian's (4) inferences that the staircase phenomenon does not occur in frog's nerve-free muscles contracting isometrically are not confirmed in cat's nerve-free (curarized) muscles. It is found to be present in these muscles when they are contracting either isometrically or isotonically. In the cat it makes no difference whether the muscle is direct loaded or after loaded. (See fig. 7.) Nor does the degree of after load influence the "treppe" phenomenon except that when the after loading screw is high so that the muscle shortens considerably before it begins to lift the load, as in figure 7 at *2*, the staircase appears to be less steep and requires possibly a slightly longer time for its development. These results do support Adrian's (4) finding upon the cardiac muscle of frogs.

It seems therefore that the staircase phenomenon in the cat's skeletal muscle is not merely an apparent increase in height of contraction ("scheinbare Erregbarkeitssteigerung") due to a prolonged contraction phase, but a genuine increase in the height of contraction due to increased irritability brought about by functional activity.

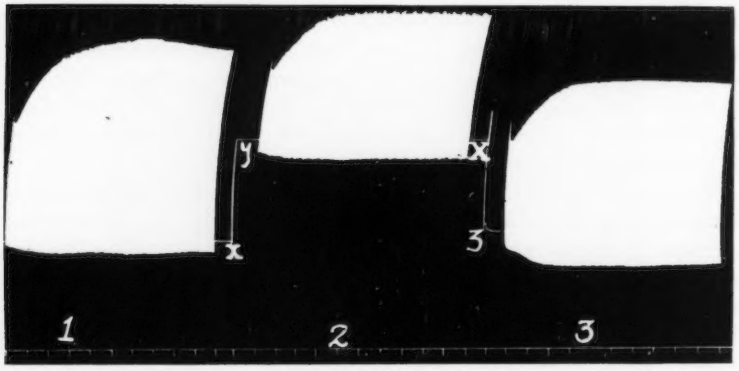


Fig. 7. Anterior tibial muscle of cat, stimulated 100 times a minute. Tension of spring 70 grams with increased tension of 7 grams for each centimeter the writing lever wrote vertically on the drum's surface. *1*, Muscle barely after loaded; *2*, after loading screw raised so that the load was supported while muscle contracted from x to y at each contraction; *3*, after loading screw lowered so the muscle was directly loaded. Time in 30 seconds. All the records show the "introductory contractions" and "treppe" phenomena.

SUMMARY

1. The staircase phenomenon as found in cat's skeletal muscle is not due to the inertia of levers, nor due to a prolongation of the contraction phase.

2. The staircase phenomenon in these muscles is due to an increase in the irritability of the muscle which is probably the result of the accumulation of small amounts of the so-called fatigue products.

3. The "treppe" phenomenon is found to be present when cat's muscles are after loaded, and direct loaded, and when contracting either isotonically or isometrically.

4. Lack of circulation through the muscle does not appreciably alter the staircase phenomenon.

BIBLIOGRAPHY

(1) For complete bibliography see articles by FRÖHLICH (3) and by LEE (2).
(2) LEE: This Journal, 1907, xviii, 267; Centralbl. f. Physiol., 1906, xx, 869.
(3) FRÖHLICH: Zeitschr. f. d. allg. Physiol., 1905, v, 295; Ergebn. d. Physiol., 1918, 47.
(4) ADRIAN: Journ. Physiol., 1920–21, liv, 15.
(5) FRÖHLICH: loc. cit., 57.
(6) FRÖHLICH: loc. cit., 69.
(7) SHERRINGTON: Journ. Physiol., 1909, xxxviii, 382.
(8) JACKSON: Journ. Amer. Med. Assoc., 1911, lvi, 1705; Experimental pharmacology, 1907, 436.
(9) LEE: This Journal, 1898, ii, xi; Arch. f. d. gesammt. Physiol., 1905, cx, 400.
(10) ROLLETT: Arch. f. d. gesammt. Physiol., 1898, lxxi, 209; Centralbl. f. Physiol., 1899, xiii, 721; ibid., 1900, xiv, 435.

THE ASH OF HUMAN SWEAT PRODUCED BY HEAT AND WORK

G. A. TALBERT

From the Hull Physiological Laboratory, University of Chicago

Received for publication October 21, 1922

Upon the examination of the literature I have found that scarcely any work has been done on the determination of the ash of the sweat. Smith (1) and Pugliesse (2) have given us reports on the ash from the horse, while Camerer (3) seems to stand quite alone as far as any such work has been done on man. Even his work has been confined to sweat produced by heat. Pugliesse, however, studied both heat and work sweat of the horse. As far as determining the constituents of work sweat of man is concerned, this seems to have been first attempted by Cohnheim and Kreglinger (6) and later by Viale (4), who confined themselves to sodium chloride elimination. The latter was the first to make a comparison of the human heat and work sweat.

Favre (5) found that of the inorganic substances the chlorides furnished by far the largest amount, particularly the sodium chloride, while the sulphates were exceedingly small and the phosphates were only a trace. So any important fluctuations in the ash could be reasonably attributed to the sodium chloride.

As far as I have been able to discover, no work has been done on the ash of the human work sweat. My purpose then was to compare the ash produced from work with that produced from heat under the same environmental conditions, as far as possible, with the exception of temperature. So for that purpose I employed the same rubber jackets for the chest, arm and leg as were used in collecting the sweat for acid determinations (11). In a similar manner the stationary bicycle and the sweat cabinet were employed for producing work and heat sweat respectively.

Methods. In my experiments I used either new or thoroughly cleaned porcelain crucibles which with their covers were heated and weighed, then the operation was repeated until no loss of weight could be detected. These crucibles were then kept in desiccators until time to receive the sweat.

Into these crucibles 10 cc. of filtered sweat were accurately pipetted and weighed. After deduction of the weight of crucible it would give the weight of this amount of sweat, which being compared with an equal volume of distilled water, with temperature correction, would give a difference that would represent the total solids. The sweat was then evaporated over a water bath and subsequently ashed with the usual precautions.

Heat and work sweat. I performed twenty experiments, ten of which were samples taken from heat sweating and ten from work sweating. In table 1 there was an average ash of 0.337 per cent from heat sweat and 0.443 per cent from work sweat. These results are quite in harmony with the determinations of Pugliesse on the horse. They also accord very well with the results obtained by Viale on the sodium chloride content of man in comparing heat and work sweat. The latter claimed that there was a gradual increase in this salt as the work progresses, and in fatigue he has found as high as 0.85 per cent, while in two heat experiments he obtained as low as 0.154 per cent and 0.135 per cent respectively.

Camerer claimed to have found from electric light bath a sodium chloride content as high as 0.66 per cent, hot air bath 0.78 per cent, and a vapor bath of only 0.465 per cent. These figures seem high when compared with my ash determinations. However, my figures are not so far from those obtained by him in the vapor bath. In fact, the conditions under which sweat was secreted under a rubber jacket would be much like a vapor bath.

In connection with the increase of sodium chloride in the sweat as a result of long marches on a mountain, Cohnheim and Kreglinger (6) have spoken of an enormous diminution of the salt in the urine on the following day, and again when sweating is profuse on a march, the gastric juice may be deprived of its hydrochloric acid.

Cohnheim, Weber, Kreglinger and Tobler (7) state that the loss of weight which frequently follows a fatiguing march may be quickly compensated for if a chloride diet is given. Which proves that in fatigue there is a general chloride insufficiency in the organism.

It is well to note on the contrary Ardin-Delteil (12) has discovered that in heat sweating there is a progressive diminution in NaCl content. Also Tarugi and Tomassi (13), who provoked a profuse sweating, found that the viscosity and molecular concentration diminished in the time in which the sweat was emitted under the action of heat.

With the increasing elimination of sodium chloride from the sweat glands in fatigue, the question naturally arises as to the state of the blood and tissues, especially the muscles under these conditions. Unfortunately, on this point there has not been an entire unanimity of opinion.

Rogozinsky (5) tells us that the physical and chemical properties of the blood do not change, but the muscles, on the other hand, lose water as a result of work.

Buglia (9) reports that the molecular concentration of the blood undergoes a slight increase, while the electric conductivity remains the same in fatigue, while in the muscles the osmotic concentration diminishes as the conductivity diminishes.

Gerhartz (10) found a diminution of water in the blood as a result of prolonged work, also an increase in specific gravity, hemoglobin and nitrogen, while in muscles less water and less mineral substances.

On the other hand, Cohnheim, Kreglinger, Tobler and Weber found as a result of fatigue on Mt. Rosa at 3000 meters a diminution of red corpuscles and hemoglobin. Viale in two experiments confirmed their results.

Here we have a fairly good agreement as to changes brought about in the muscles, but widely divergent as to the blood as result of work.

It seems quite certain that there is a depletion of the water reserve and also of the NaCl in the tissues. As to the former, it may be assumed that the loss of water to the muscles is due to the requisition of the sweat glands and the latter are stimulated by the heat produced from the metabolism of the muscles. We have no assumption that the increased salt elimination is directly due to the metabolism. On the contrary, it may be assumed the increased water output acts as a vehicle in removing more salt.

It is difficult to put an interpretation on the wide variance in their results on the blood.

It seems to me the problem is too complicated to do much theorizing in the light of our present knowledge. This much might be said, however, while there might be a temporary dilution of the blood at a certain stage of the activity of the muscles, yet, on the contrary, it seems more reasonable that in a long-continued fatigue, and provided there is no intake of water, there would be such an exhaustion of the water from the tissues for the purpose of heat regulation as to cause a concentration of the blood.

As to the increased of NaCl in work sweat we may find part of our solution in the decrease of the salt in the urine, and also of the HCl in the gastric juice as determined by Cohnheim and Kreglinger. Furthermore, part of the concentration can likely be laid to the fact of the increased respiration which would have a tendency to carry away more water through the lungs.

Regional variations. One of the most interesting points developed in the study of the ash is the regional variations on the covered parts of the body.

In table 2, comparing the sweat which was taken simultaneously from covered arm and covered leg, the former shows an average of 0.375 per cent of ash, and the latter shows an average of 0.262 per cent. In the third column the bare leg yields 0.219 per cent.

Similar results are noted in table 3. In this case, however, the sweat was collected from only two parts of the body.

In table 4 the covered arm and covered chest are compared in like manner. Here the covered arm gave an average ash of 0.356 per cent, while the chest was 0.438 per cent.

In table 5 the average ash from the bare chest and from the covered leg were compared. The former yielded an average ash of 0.368 per cent, the latter 0.234 per cent.

Comparison of naked and covered skin. Another point of interest is the difference in the amount of ash obtained from the naked parts compared with the covered parts. In table 2 eight experiments were with the covered leg and bare leg, and it will be noted without exception that the former gave the greater ash. The same point is brought out in table 6, where we compare the covered chest with the bare chest. These samples could not be obtained by simultaneous sweating like that of the leg. However, the differences in the averages are so great that the comparisons become quite as convincing. So it is observed that with corresponding parts of the body the covered portion gives a greater ash than the naked.

In table 5, when comparing different parts of the body, as bare chest and covered leg, we observe that the regional factor may predominate, as seen here where the ash average for the bare chest is 0.368 per cent, while covered leg is 0.234 per cent.

It is of interest to note that the parts of the body that eliminate the most acid yield the smallest ash in the sweat. Particularly is this true of the leg. The chest as a rule eliminates the smallest amount of acid and the greatest amount of ash when the parts are covered. However,

the difference in the acidity of the arm and chest is not as marked as the difference in the ash.

The results that we have obtained from the covered parts over against the uncovered are different from what we would naturally expect. We would with some reason suppose that there would be a condensation of the insensible perspiration under the jackets which would have a tendency to dilute the sensible perspiration.

Professor Carlson suggested that the skin temperature might be an important factor in raising the salt content of covered parts. To that end I tried out a series of experiments on myself. I chose two hot July afternoons with a room which allowed the full rays of the sun. I stripped off all clothing and lay on my back on a bed and tested the skin temperature with ordinary clinical thermometers comparing particularly covered and uncovered parts simultaneously, also choosing parts of the body where it was possible to cover the bulbs of the thermometers completely to the exclusion of the air. For this purpose I chose the popliteal space, the antecubital, the axilla and the abdomen. In the last named position the thermometers were imbedded in the creases of the skin produced by bending forward. Thermometers were standardized before using and the room temperature varied from 105° to 108°F. Mouth temperature was 99°F.

In table 7 are the comparative temperatures in the popliteal region taken simultaneously from the covered leg and the bare leg. Each test lasted ten minutes. Here will be found a higher temperature of 0.5°F. with the covered leg. Test of space at the antecubital was taken at the same time. The covered arm and covered leg run practically the same. The first five experiments were performed on one day and the last five the next day.

In table 8 similar tests were tried at the antecubital fossa with the same general results except that the differences were not so marked. Likewise, the results in table 9. In table 10 we found that the differences were even more pronounced than from the leg.

I offer this data as a possible factor in increasing the salt secretions on the covered parts.

Series I

TABLE 1

Ash from covered chest

HEAT SWEAT	WORK SWEAT
per cent	*per cent*
0.306	0.433
0.301	0.491
0.310	0.407
0.317	0.400
0.541	0.691
0.380	0.370
0.291	0.410
0.366	0.301
0.298	0.420
0.388	0.500
0.337 Av.	0.443 Av.

TABLE 2

COVERED ARM	COVERED LEG	BARE LEG
per cent	*per cent*	*per cent*
0.537	0.264	0.232
0.354	0.297	0.364
0.343	0.310	0.210
0.324	0.259	0.184
0.439	0.208	0.204
0.325	0.288	0.239
0.266	0.203	0.190
0.343	0.270	0.220
0.375	0.262	0.219

TABLE 3

COVERED ARM	COVERED LEG
per cent	*per cent*
0.537	0.264
0.354	0.297
0.345	0.259
0.324	0.208
0.439	0.288
0.325	0.225
0.387 Av.	0.273 Av.

TABLE 4

COVERED ARM	COVERED CHEST
per cent	*per cent*
0.324	0.433
0.367	0.491
0.384	0.407
0.309	0.400
0.511	0.691
0.307	0.370
0.358	0.410
0.290	0.301
0.356 Av.	0.438 Av.

TABLE 5

BARE CHEST	COVERED LEG
per cent	*per cent*
0.318	0.203
0.363	0.243
0.422	0.255
0.368 Av.	0.234

TABLE 6

COVERED CHEST	BARE CHEST
per cent	*per cent*
0.433	0.318
0.491	0.363
0.407	0.422
0.400	0.354
0.691	0.365
0.370	
0.401	
0.301	
0.438	0.364

Series II

TABLE 7

Popliteal skin, temperature, Fahrenheit scale

	COVERED LEG	BARE LEG	COVERED ARM
First day	97.5°	97.5°	
	98.2°	97.4°	98.3°
	98.2°	97.4°	98.3°
	98.3°	97.4°	98.3°
	98.3°	97.3°	98.3°
Second day	98.7°	98.4°	
	98.8°	97.0°	
	97.9°	96.9°	97.8°
	97.8°	96.8°	97.8°
	97.8°	96.8°	98.0°

TABLE 8

Antecubital temperature, Fahrenheit scale

COVERED ARM	BARE ARM
97.8°	97.5°
98.8°	97.6°
98.5°	98.2°
98.6°	98.4°
98.6°	98.4°

TABLE 9

Axillary skin temperature, Fahrenheit scale

COVERED ARM	BARE ARM
98.6°	98.0°
98.7°	98.3°
98.7°	98.3°

TABLE 10

Abdominal skin temperature, Fahrenheit scale

COVERED ABDOMEN	BARE ABDOMEN
98.4°	96.8°
98.2°	97.3°
98.2°	96.7°
98.2°	97.3°
98.2°	97.3°

SUMMARY

1. There is a greater amount of ash in work sweat than in heat sweat.

2. There is a regional variation in the ash where the parts are covered, smallest percentage from the leg, then followed by the arm, while the largest comes from the chest.

3. There is a greater ash from the sweat of covered parts than corresponding naked parts.

4. The concentration of the salts in the sweat glands is in part a function of the temperature of these glands. The higher the temperature, the greater the concentration of the salts.

I wish in this connection to thank Professor Carlson for the inspiration and helpful suggestions received.

BIBLIOGRAPHY

(1) SMITH: Journ. Physiol., 1890, xi, 497.
(2) PUGLIESSE: Arch. Ital. de Biol., 1910, lviii, 2.
(3) CAMERER: Zeitschr. f. Biol., 1901, xli, 271.
(4) VIALE: Arch. Ital. de Biol., 1913, lix, 269.
(5) FAVRE: Compt. rend., 1852, xxxiii, 721. Vol. 35
(6) COHNHEIM AND KREGLINGER: Zeitschr. f. Physiol. Chem., 1909, lxiii, 413.
(7) COHNHEIM, WEBER, KREGLINGER AND TOBLER: Zeitschr. f. Physiol. Chem., 1912, lxxviii, 62.
(8) ROGOZINSKY: Biochem. Zeitschr., 1906, i, 207, 227.
(9) BUGLIA: Biochem. Zeitschr., 1907, vi, 168.
(10) GERHARTZ: Pflügers Arch., 1910, cxxxiii, 397.
(11) TALBERT: This Journal, 1922, lxi, 493.
(12) ARDIN-DELTEIL: C. R. de l'Acad. des Sci., 1900, cxxx, 844.
(13) TARUGI AND TOMASSINELLI: Arch. di Fisiol., 1908, v, 581.

THE INADEQUACY OF OUR PRESENT BLOOD VOLUME METHODS

PAUL D. LAMSON AND S. M. ROSENTHAL

From the Pharmacological Laboratory, Johns Hopkins University

Received for publication October 28, 1922

A review of the articles appearing under the heading "Blood Volume," since 1920, shows some twenty papers, and there are undoubtedly many more reports of investigations in which blood volume methods have been used. The latest and most comprehensive review of these methods is that by Erlanger (1). Whipple (2) and his associates have undertaken perhaps the most extensive experimental tests of the methods. Plesch (3) has recently published a large series of experiments in which the blood volume has been determined in normal and pathological conditions. The reader is referred to these articles for the general literature of the subject. The other papers referred to above deal chiefly with refinements of technic, but offer no change in the fundamental principles of blood volume determination and will not be dealt with here.

Although so called "new" methods appear from time time, they all fall into the four old classes of:

1. The Welcker method, in which the volume is determined from a comparison of the total hemoglobin found by extraction, with the hemoglobin content of a known volume of blood.

2. Plasma soluble methods, in which a known amount of some substance, soluble in the plasma, is intravenously injected and from its dilution in the blood the volume calculated.

3. The carbon monoxide method, where a known amount of carbon monoxide is inhaled, and from its dilution in the blood the volume calculated.

4. Relative volume methods, in which change of volume is determined by variation in concentration of some substance in the blood.

Each of these methods has its supporters who believe it superior to the others, each having his own particular way of using the method, which he also believes to be the best. There is little agreement as to

which method is the best, but there are ardent upholders of some blood volume method. It is also of interest that the strongest supporters of blood volume methods are those who have done most work with them. Erlanger in his review is quite impartial, points out the various sources of error in the different methods and draws no conclusion as to their value. However, in a paper by White and Erlanger (4), his belief in the value of the methods is shown by the following statement: "The persistent increase of blood volume, 2 or more per cent, is less than the amount of water which must be added to . . . ." Plesch is of course a firm believer in the value of the methods. Whipple and his associates, although gradually finding out the errors of the methods, use as part of the title of the seventh paper in their series of blood volume papers, "Accurate Estimation of Absolute Blood Volume," and on the first page of this paper state, "In brief, it is our conviction that the *dye or hemoglobin methods give the true plasma volume* (4.8 cc. per 100 grams of body weight), that the *carbon monoxide method gives the true body hemoglobin volume* (4.2 cc. R. B. C. per 100 grams body weight), and finally that the true blood volume is the sum of these two figures (9 cc. per 100 grams of body weight)" (5).

From the work and conclusions of these three workers, (chosen because most widely read on account of recent work), and many others the experimenter, interested solely in the determination of blood volume in the condition at hand, may be led to believe that the methods are adequate for his purposes, and may proceed to accumulate data and draw conclusions from their use. The purpose of this paper is to determine whether or not he is justified in placing reliance on these methods.

In 1920 Lamson and Nagayama (6) published a paper on blood volume methods in which they concluded from several years of experimental work, "It has been shown that the true total blood volume, total plasma volume, or total red cell volume cannot be measured by any of our present methods." Erlanger (7), in his review, in reference to the above paper says, "While admitting the soundness of their underlying argument, it has yet to be demonstrated that, excepting, possibly, certain very abnormal conditions, the two types of blood volume methods necessarily give inconsistent results and, if they do, that the discrepancies are due to uneven distribution and uneven size of the red cells." Although an uneven distribution of red cells was only one of the errors mentioned, it is of interest to note that Whipple a year or so later with no reference to Lamson and Nagayama's paper, after

accepting the plasma soluble method, publishing an improvement on it, and using it for experimental purposes, finally in his seventh paper discards this method and the carbon monoxide methods for exactly this same reason. He says "We believe other investigators have fallen into error due to *calculation of the total blood volume* based on the red cell haematocrit—usually 50 per cent in dogs. This gives erroneous figures . . . ." "This error comes from the assumption that the blood cells and plasma are uniformly mixed in all parts of the circulating system" (8).

Lamson and Nagayama after first showing implicit confidence in blood volume methods, and one of them (L) publishing papers based on results obtained by these methods, gradually came to the conclusion that we have no method by which the blood volume can be accurately determined. They pointed out that each method has a fundamental error, and therefore could not be counted on. Their work has been criticised as being of theoretical rather than of practical importance, on the basis that the experimental difficulties encountered by them were in too abnormal conditions. In this relation it is of interest to note that Whipple discards the only two practical methods which have been developed on account of these errors, and we feel that we now have experimental evidence which shows that the methods are not only theoretically but practically untrustworthy.

The important fundamental errors of the methods will be found to fall into two groups: *a*, red cell distribution, and *b*, mixing time and elimination time.

Red cell distribution. All three methods, the Welcker, the plasma soluble methods and the carbon monoxide method, are dependent upon taking a sample of blood for analysis which is assumed to be a true sample of all the blood in the body. We have no proof that this assumption is correct as it is at present impossible to obtain the concentration of the entire blood. As all our methods depend upon such a sample, there is no proof that the volumes found are true volumes. They can be so by chance only. On the other hand there is gradually more evidence accumulating to show an uneven distribution of red cells, especially in pathological conditions, which are the conditions where blood volume determinations are of great interest. Cannon (9) has shown marked variation between superficial and deep counts in conditions of shock, Bostrum (10), in certain experimental conditions, Duke (11) in pernicious anemia. In such conditions a sample from one part of the circulation, as a great vein, might give very misleading results.

Mixing time and elimination time. Except for the Welcker method, which cannot be used without the destruction of the individual, all methods depend upon some substance being taken into the blood stream, and *mixed evenly* with the whole blood *before any of the substance is lost.* There has been a great deal said about this mixing and elimination but in spite of the obvious uncertainties, investigators have gone ahead as if all were well. Concentration curves have been plotted, all of which show a decided fall in concentration after the first few minutes, and some irregularity before this. None of the curves show an establishment of equilibrium for at least thirty minutes or more. The general *assumption* has been that during the first few minutes mixing took place, and that after this elimination began, and on this account samples have usually been taken between three and five minutes after injection of the substance used. If we ignore for the present unknown probabilities, and deal only with what we can prove, we find that *in the first place we have no knowledge of the absolute volume, and therefore are unable to determine the error of any method.* Certain observers in this field have made a false calculation of error. A series of determinations on one individual has been carried out showing, let us say, a 5 per cent variation. The error of the method has then been spoken of as 5 per cent. This is of course simply the variation of separate determinations and not the error of the method. It is similar to finding 8 to 10 per cent sugar in a solution and calling the error 2 per cent when the actual amount of sugar was 40 per cent. To obtain the true blood volume there must be complete mixing and no loss of substance. Here we have two unknowns. Any concentration curve will be the resultant of these two elements, and it is impossible to tell at any one point on the curve whether the per cent found is due to complete mixing, and no loss, complete mixing and partial loss, incomplete mixing and no loss, or incomplete mixing and partial loss. The blood volume found is directly dependent upon the concentration at the time chosen for the sample, and there is no justification whatever for taking a sample before equilibrium has been reached.

Erlanger (12) has summarized the mixing curves obtained by different observers using a variety of substances. These curves are all more or less similar except one obtained by Harris (13) who plotted the concentration of congo red at thirty-second intervals after injection. He obtained a smooth rounded curve with its peak one and one-half minutes after injection, and from this concluded that the time for taking the sample was at the one and one-half minute point, and used

this in obtaining a factor for the correction of blood volumes. As far as we can see, Harris used only one dog and made one observation only. As so much importance has been given this one experiment by recent writers, it was thought worth while to repeat the experiment using vital red. As will be seen in figure 1, no uniform curve of mixing could be obtained during the first few minutes after injection. We feel

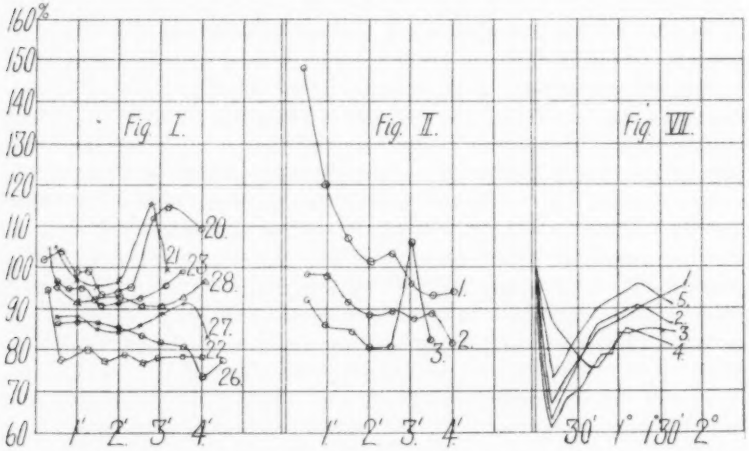


Fig. 1. Dogs only were used, anesthetized with ether, a tracheotomy done, and an ether bottle attached by which it was possible to obtain a very uniform anesthesia. After three-quarters of an hour under ether to insure uniform conditions a sample of blood was drawn from one jugular vein previously exposed. The dye was then injected through this vein, and at 30 second intervals samples of blood were drawn from the opposite jugular vein from a large needle using a glass syringe. A standard plasma dye preparation was made according to Keith, Rowntree and Geraghty, and the samples compared with this. The following are the concentration curves obtained in seven dogs.

Fig. 2. These three curves are concentrations of vital red plotted in the same way as in figure 1, only after the injection of a large dose of epinephrin intravenously (0.9 mgm. per kgm.). It was thought that on account of a change in the circulation rate very different curves might be obtained. One curve is very striking but the others are not much different than before the injection of epinephrin.

Fig. 7. These curves are plotted as actual concentration of plasma proteins in curves 1, 2 and 3, and of vital red 24 hours after injection when it had come to equilibrium, in curves 4 and 5. They show the change of concentration of these substances after the injection of 0.8 per cent saline and Armour's Pituitrin. The inverse of these curves would represent volume change and when compared with figure 5 it will be seen that they correspond closely with hemoglobin concentration, but are *the opposite of plasma volume curves.*

that this experiment shows the absurdity of taking samples at exactly three or four minutes after injection as has been the custom in the plasma soluble methods. By consulting this figure it is easy to see the differences in volume which would be obtained by using a three or four minute time, and the difference in volume between the different animals using the same time. This irregularity of mixing may account for the great variation in volumes found in normal dogs by Whipple in spite of his very carefully controlled technic, 100 per cent by the carbon monoxide method, and 82 per cent by the dye method (14).

We see then that blood volume methods are based on nothing but unknowns. The Welcker method on the unknown relation between the red cell concentration of the sample taken to the whole blood. The plasma soluble methods, and the carbon monoxide method, on this same unknown, and also on the unknown mixing time and unknown amount of elimination. There is no known mathematical method by which an equation made up of nothing but unknowns may be solved, and we feel justified in once more stating that it is impossible by our present methods to determine the blood volume, plasma volume or red cell volume.

Examples of the incongruity of results obtained by methods in use are the following.

Divergence of results with two blood volume methods. Epinephrin given in large doses to dogs causes a decrease in plasma and blood volumes by the vital red method, and an increase in the hemoglobin concentration showing a decreased volume by this means also (15). In this condition the two methods give similar results.

Epinephrin given in large doses to rabbits, however, causes a similar decrease in plasma and blood volume by the vital red method, but no change in the hemoglobin concentration. Here the two methods show divergent results.

October 31, 1919: Rabbit, 2 kgm.
10:46. Red cells 6,837,000. Plasma volume 81.6 cc.
10:52. Red cells 7,052,000.
10:57. Epinephrin 2 mgm. intravenously.
11:12. Red cells 6,625,000.
11:15. Red cells 6,837,000. Plasma volume 63.4 cc.

Finally if a clamp is put on the hepatic artery of a dog and epinephrin given intravenously, there will be a decrease in plasma and blood volumes by the vital red method, but no change in the hemoglobin concentration. If an hour or two later the clamp is removed from the hepatic artery, there will be an increase in hemoglobin concentration

indicating a decrease in blood volume but no change in volume by the vital red method will be found (16).

We may then have the plasma volume and blood concentration run parallel, the plasma volume decrease and the hemoglobin concentration remaining constant, or the plasma volume remaining constant and the hemoglobin concentration increasing.

Relative volumes. Experiments have been carried out in order to determine the effect of different substances upon the rate of fluid loss from the circulation under certain conditions. These experiments are to be reported in full later, but the impossibility of interpreting results from any one method is shown by the utter lack of agreement in two accepted blood volume methods, namely, the vital red method and the hemoglobin concentration.

These experiments were all carried out as follows. Dogs were etherized using a tracheotomy tube and ether bottle. This gave a constant anesthesia which needed no attention when once under way. Both jugular veins were exposed by a buttonhole incision through the skin. After allowing about an hour's etherization to establish conditions of equilibrium, samples of blood were taken with a small syringe and needle from one jugular vein, and placed in test tubes with a small amount of sodium oxalate. One-half a cubic centimeter of this blood was diluted in 100 cc. of dilute hydrochloric acid, allowed to stand one hour, and read in a Dubosq colorimeter. The first sample was used as the standard and called in each case 100 per cent. Injections were made through a cannula tied into one femoral vein.

Vital red volumes were determined by the method of Keith, Rowntree and Geraghty (17). Samples of blood were taken from the jugular vein and injections made in the opposite vein. Care was used to take blood exactly three minutes after the injection of dye. On account of the presence of dye in the blood both determinations could not be carried out on the same animal.

Changes of volume have been studied by use of the above mentioned methods and also by changes in concentration of some substance in the blood. The hemoglobin, red corpuscles, plasma proteins, total solids, electrical conductivity, freezing point, etc., have been used as indices of concentration change, water content or volume change. Each has its drawbacks. Hemoglobin or red cell concentrations have the advantage that these do not pass out of the vessels, but on account of changes in distribution throughout the circulation may give misleading results. All diffusible substances may pass in or out of the vessels, and non-

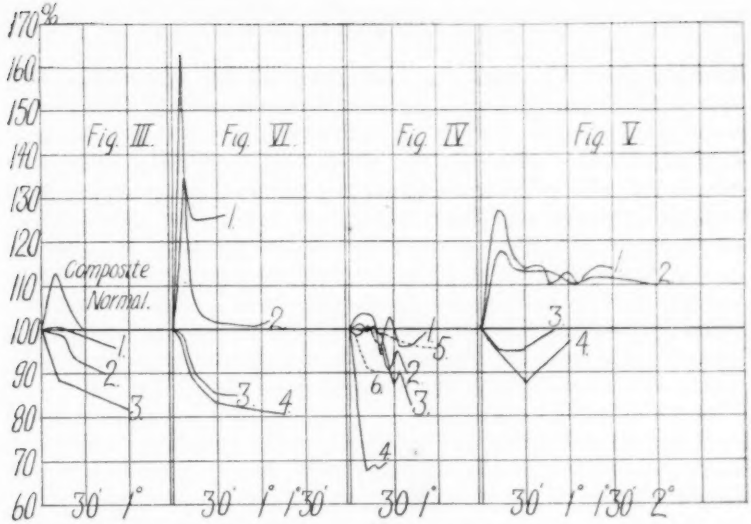


Fig. 3. The following series of curves is shown to compare a curve of volume change represented by plasma volume determinations done by the Keith, Rowntree and Geraghty method, and volume change as indicated by plotting hemoglobin concentration. All of these experiments were carried out on dogs under ether anesthesia, and blood samples taken from the jugular vein. The hemoglobin concentration was determined by drawing about 0.5 cc. of blood with a small needle and syringe, measuring an exact amount of this (0.4 cc.) and diluting in 100 cc. of dilute HCl. After standing one hour the solutions were read in the Dubosq colorimeter using the first as the standard.

The upper curve is a composite of hemoglobin concentrations after the injection of 25 cc. of 0.8 per cent saline per kgm. intravenously in 10 minutes, in eight dogs. (See Lamson and Roca: Journ. Pharm. Exper. Therap., 1921, xvii, 483.) The curves are the inverse of the actual concentrations to express blood volume change. The lower three curves are volumes determined by the dye method at intervals after the injection of the same amount of salt. It will be seen that although there is uniformity of each method *the two methods give exactly opposite results*.

Fig. 4. Shows six curves all of which show a downward trend representing a decrease in blood volume. These curves were obtained by the plasma volume method in curves 5 and 6, and the hemoglobin method in 1, 2, 3 and 4. In this experiment varying amount of histamine phosphate was added to the salt solution injected, and in this condition the two methods *agree*.

Fig. 5. These curves were obtained by the dye and hemoglobin methods after the addition of large amounts of Armour's Pituitrin to the salt solution injected. Curves 1 and 2 were plotted by the hemoglobin method and 3 and 4 by the dye method. It will be seen here again that *the two methods give opposite results*.

Fig. 6. In this experiment 5 grams glucose per kilo were intravenously injected in a concentrated solution, and the volume curves plotted as before by the dye and hemoglobin methods. It will be seen that there is an enormous increase of volume by the hemoglobin method but a decrease by the vital red method. Here again *the two methods give opposite results*.

diffusible plasma proteins can easily pass through the walls of the liver capillaries and come into the blood from the tissues. The plasma proteins on account of their non-diffusibility and the ease of their determination by refractometric means, have probably been most extensively used. We have used another substance for a concentration method of determining volume change, that is, vital red. Twenty-four hours after injection the concentration of this substance has come to an apparent equilibrium in the blood, and sudden changes in concentration produced by experimental means may be of interest as a possible indication of volume change, but with the limitation that the dye may leave the circulation, or possibly might be reabsorbed. It can then be used as an index only, and not as absolute proof of volume change.

In the following experiments it is seen that the plasma proteins and dye concentration curves run practically parallel after the injection of saline and pituitrin, and by comparison with figure 5 it will be seen that they are similar to the hemoglobin concentration curves which had to be done on separate animals. Here we have three methods giving practically identical results but quite different from those obtained by the vital red method.

CONCLUSIONS

It has been shown that blood volume determinations depend upon the accuracy of the sample taken, thoroughness of mixing and degree to which the substance injected remains in the circulation during the time taken for mixing. We are at the present time unable to determine accurately any of these three factors.

Conditions have been produced experimentally by which these factors determining "blood volume" have been varied, causing two standard "blood volume" methods to give widely divergent results, and showing the impossibility of determining which, if either, is the true blood volume, by the use of the method alone.

Concentration curves of vital red have been plotted during the first five minutes after injection which show very great variation in form. From these curves it is evident that the choice of a three minute or other time for mixing is a purely arbitrary choice; that the volume found will vary with the time at which the sample is taken and with any variation in time of mixing in the individual case.

On account of this irregularity of mixing time, results obtained from samples taken at a fixed time after injection are incomparable in different individuals, which may account for the great variation found in some subjects.

This also shows the worthlessness of a volume determination on a single subject for comparison with the normal, derived from the average of many.

Constant and accurate figures may be obtained by blood volume and plasma volume methods which are the blood or plasma volume by chance only, and a variation in the "volume" found by these methods may equally well mean a variation in mixing, red cell distribution or rate of loss of substance used.

Finally these methods should by no means be discarded as valueless, as a series of determinations may be of great diagnostic importance both experimentally and clinically, showing constant values or variations in different conditions. They should however not be interpreted as necessarily true volume changes.

BIBLIOGRAPHY

(1) Erlanger: Physiol. Reviews, 1921, i, 177.
(2) Smith, Arnold and Whipple: This Journal, 1921, lvi, 336.
(3) Plesch: Zeitschr. f. klin. Med., 1922, xciii, 241.
(4) White and Erlanger: This Journal 1920, liv, 7.
(5) Smith, Arnold and Whipple: This Journal, 1921, lvi, 336.
(6) Lamson and Nagayama: Journ. Pharm. Exper. Therap., 1920, xv, 331.
(7) Erlanger: Physiol. Reviews, 1921, i, 195.
(8) Smith, Arnold and Whipple: This Journal, 1921, lvi, 360.
(9) Cannon: Journ. Amer. Med. Assoc., 1918, lxx, 526
(10) Bostrum: This Journal, 1921, lviii, 195.
(11) Duke: Arch. Int. Med., 1922, xxx, 94.
(12) Erlanger: Physiol. Reviews, 1921, i, 193.
(13) Harris: Brit. Journ. Exper. Path., 1920, i, 147.
(14) Smith, Arnold and Whipple: This Journal, 1921, lvi, 352.
(15) Lamson: Journ. Pharm. Exper. Therap., 1915, vii, 169.
(16) Lamson and Keith: Journ. Pharm. Exper. Therap, 1916, viii, 247.
(17) Keith, Rowntree and Geraghty: Arch. Int. Med., 1915, xvi, 547.

NOTE ON THE SHAPE OF THE GLOMERULI AND BOWMAN'S CAPSULES IN THE ACTIVE AND INACTIVE KIDNEY

W. N. F. WOODLAND

From the Wellcome Bureau of Scientific Research, London, England

Received for publication November 6, 1922

In a paper "On Changes in the Glomeruli and Tubules of the Kidney Accompanying Activity" by T. G. Brodie and J. J. Mackenzie, published in 1914,[1] experimental evidence is adduced to prove that whereas in a condition of "rest" "the glomerular surface always lies in contact with the capsule wall" in diuresis or, to use the authors' expression, "after activity," "the glomerulus stands away clearly from the capsule wall . . . . a considerable accumulation of secretion being found between them," and the conclusion is drawn that "all the appearances found are explained as resulting from the action of a high pressure in the fluid *secreted by the glomerular epithelium*."[2] Since this and similar statements made by these authors are likely to be regarded as well founded, it is necessary, in view of the fact that during diuresis under normal conditions the glomerulus is *not* separated from the outer wall of the Bowman's capsule, that these statements should be corrected, and it is the purpose of this note to demonstrate the fact I have just mentioned. I also propose to show that there exist two other factors, both capable of producing, and which in fact did produce, the separation of the glomerulus from the outer wall of the Bowman's capsule observed by Brodie and Mackenzie. The first of these factors is the sudden drop in the blood pressure in the glomeruli which occurred in all the experiments of Brodie and Mackenzie, combined with "the action of a high pressure in the fluid secreted" (not by the glomerular epithelium but, as I believe, by the glandular walls of the tubules); in other words, the fluid secreted by the tubules entered into the Bowman's capsules because the empty spongy glomerulus offered no resistance and because secretion pressure was high. Brodie and Mackenzie admit the existence of this factor when they refer to the fact

[1] Proc. Royal Soc. London, Series B, lxxxvii, p. 593.

[2] My italics.

that they "were never able to keep the blood in a kidney that was excised at the height of activity. At the instant of excision such a kidney is hard and tense, and instantly becomes soft when the first ligature is tied round the pedicle. This is even the case though the vein be first ligatured," and when they comment upon "the great effect of obstruction [ligature of the ureter in the active kidney] upon the distension of the capsule and accumulation of fluid within the capsule."

The second of the two factors is the differential contraction of the tissues in a kidney (or any other organ) exposed to the heat of an embedding bath and to the action of alcohols and clearing reagents, when the organ is embedded for section-cutting. The spongy thin-walled glomeruli differ greatly in consistency from the thick glandular walls of the tubules and the former will obviously contract to a much greater extent than the latter during embedding, and since the outer walls of Bowman's capsules are more or less attached to the walls of surrounding tubules, the fact that a space appears between these outer walls and the glomeruli in sections of wax-embedded kidney is not surprising. That this differential tissue-contraction does in part account for the results obtained by Brodie and Mackenzie I have proved by experiment (vide infra).

It follows from what has just been said that if we wish to ascertain the condition of the glomeruli in Bowman's capsules during kidney activity, we must avoid *1*, the lowering of the glomerular blood pressure when fixing and preserving the kidney tissues; and *2*, differential contraction of the tissues owing to heat or reagents. Diminution of the glomerular fluid pressure may be avoided by perfusion of the kidney with saline, fixation of the kidney tissues being effected by substituting a fixative for the saline after the kidney has become active. The kidney is thus fixed while active without lowering of the arterial fluid pressure. Differential tissue contraction may be avoided by macerating the fixed kidney in some fluid which does not contract the tissues. Both of these conditions were observed in the experiments (performed in India) now briefly to be described, and it is to be remarked that the results of these experiments prove conclusively that during diuresis the glomerulus completely fills the Bowman's capsule, and that only when the glomerular fluid pressure was caused to fall below the secretion pressure does fluid enter the capsule, i.e., does the glomerulus separate from the outer wall of the capsule.

In these experiments the large Indian frog, *Rana tigrina*, was employed. Th animal was anesthetized with ether, the cerebrum removed, and the kidneys, ureters and all the large arteries and veins well exposed from the dorsal surface.

Experiment 1. A cannula (which perfused 47.5 cc. of fluid per minute at 24 cm. pressure of water) was tied into the coeliaco-mesenteric artery, and 0.6 per cent saline, plus 40 cc. fresh human urine and 1 gram Na_2SO_4 added to each 2000 cc. of the saline, was perfused at 24 cm. pressure. The heart was cut out to allow of the escape of the fluid and both iliac arteries were ligatured to raise the arterial pressure and so ensure vigorous kidney secretion. The ureters were cleared from adjacent tissues and inserted in collecting tubes supported on pads of putty.

Perfusion was started at 11:40. Between 12:2 and 12:17 (15 minutes) 1.5 cc. of "urine" was collected in the two tubes, and during this period 66 cc. of the saline were perfused.

At 12:19 I quickly siphoned off most of the fluid from the perfusion bottle and substituted chromic acid fixative (0.25 per cent chromic acid, 0.1 per cent acetic acid, plus a little glycerine) which entered the kidneys at 12:22.

Between 12:25 and 12:40 one drop of yellow fluid was "secreted."

Between 12:25 and 12:40 19 cc. of the fixative perfused the kidneys.

Between 12:40 and 12:53 nothing was "secreted."

Between 12:40 and 12:53 12 cc. of the fixative perfused.

I then removed the kidneys, cut them into several pieces and placed some of these in Marcacci's fluid (equal parts of nitric acid, water and glycerine) for 24 hours (Marcacci's fluid gave much superior results as compared with other macerating fluids such as weak alcohol, dilute KOH, weak picric acid, etc.).

Experiment 2. Similar to experiment 1, save that I substituted picric acid solution (4 gm. in 2000 cc. of saline) instead of the chromic acid fixative.

The two kidneys secreted 1 cc. during the 15 minutes' perfusion with the saline-urine-sulphate solution (36 cc. perfused) but stopped "secreting" when perfused with the picric acid. Both kidneys cut up and pieces placed in Marcacci's fluid.

On the morning after each of the preceding experiments I teased up the pieces of kidney macerated in the Marcacci's fluid and found that in practically all cases[3] the glomeruli were *large and spherical and completely filled the Bowman's capsules.*

In each of the preceding experiments I also dehydrated, cleared, embedded and sectioned one portion of the kidney, and all the sections thus obtained showed a distinct *shrinkage of the glomerulus and consequently a space between the glomerulus and the outer wall of the capsule.* Differential tissue shrinkage is thus capable of explaining, at least in part, the results of Brodie and Mackenzie. I may also remark that

[3] The very few cases in which a space was discernible between the glomerulus and the outer wall of the capsule were probably due to mechanical distortion involved in the process of teasing.

the relative shrinkage of the glomerulus is not nearly so evident in sections of "resting" kidneys in which the different tissues are not expanded when the organ is fixed.

Reverse current experiments. To ascertain if a considerable space was developed between the glomerulus and the outer capsule wall when the pressure in the glomerular capillaries is artificially lowered and the secretion pressure is artificially raised, I performed two more experiments, which consisted of perfusing the frog's kidney in the direction the reverse of the normal, i.e., the perfusing fluid entered at the renal veins and escaped by the renal arteries. Under these conditions the rate of secretion is made greater,[4] pressure for pressure, than with the normal direct current, i.e., secretion pressure is very high, and the glomerular fluid pressure is obviously very reduced.

Experiment 3 (reverse current). In this experiment a cannula (with a flow of 70 cc. per minute at 24 cm. pressure of water) was tied into the post-caval vein and a solution of 0.6 per cent saline, in each 2000 cc. of which I had dissolved 1 gram of urea, was perfused at a relatively low pressure of 15.5 cm. Both renal afferent veins were ligatured and the renal arteries were cut for the escape of the fluid.

A prolific secretion being obtained, I quickly substituted chromic acid fixative for the saline-urea fluid and the kidneys became well fixed.

I may mention that whereas the fixative in the normal direct current experiments "dried up" the secretion at once, in this reverse current experiment "secretion" of chromic acid was still continuing (0.3 cc. in 15 minutes) an hour after the chromic acid had been started, and likewise the chromic acid continued to flow into the kidney.

On maceration in Marcacci's fluid all the glomeruli were observed to be greatly contracted and a large space separated each glomerulus from the outer capsule wall. The tubules were greatly swollen.

This result proves that the results obtained by Brodie and Mackenzie were primarily due to the lowering of the glomerular arterial pressure which occurred in the experiments of these authors when the kidneys were removed from the body, as already suggested. It also disproves the suggestion made to me by my friend Doctor Whitehouse that the

[4] Secretion during the reverse current is always much more profuse (in the Indian frog about eight times as much, and in the rabbit about six times as much, the pressures of the perfusing fluid remaining the same for the two currents) than during the normal direct current. Vide "On the 'Renal Portal' System (Renal Venous Meshwork) and Kidney Excretion in Vertebrata" by the present writer, published in the Journal of the Asiatic Society of Bengal, N. S. xviii, pp. 85–193, 1922, in which the reason for this fact is fully elaborated.

swelled condition of the glomeruli found in the first two experiments described above was due to the action of the acetic acid.

A similar experiment, in which picric acid solution replaced the chromic acid fixative, gave similar results.

The results of the foregoing experiments thus completely disprove the statement of Brodie and Mackenzie that during normal diuresis fluid is contained in the capsule and invalidates their conclusion that the glomerular epithelium *secretes* urine, and the only alternative suppositions are that the glomerulus either *filters* urine or has nothing whatever to do with the actual production of urine. I have elsewhere (in the paper referred to in footnote[4]) supplied at length my reasons for believing that the whole of the urine is secreted by the tubular epithelium, the encapsulated glomerulus solely being a pressure-reducing and current-retarding though volume-maintaining (in other words, has the same effect upon the blood stream as a waterfall has on a river) device necessitated by the proximity of the kidneys to the aorta—the seat of maximum arterial pressure.

RESISTANCE OF FISH TO SALTS AND ALKALINITY

R. T. YOUNG

From the University of North Dakota

Received for publication October 16, 1922

The ability of aquatic life to withstand changes in its chemical and physical environment, and the effect of such changes thereon, has been a fertile field of experiment for many years. Many are the questions involved in this problem, but few are the definite answers which we have as yet received. From both the economic and the purely scientific standpoint the problem is of large interest, involving at the same time questions of great practical importance, as well as some of those touching the most intricate processes of life. Knowledge of the resistance of fish to dissolved salts is obviously essential to rational stocking of new waters, but we have as yet, so far as the writer is aware, no standard for determining the chemical suitability of any water for a given species of fish.

Previous experiments in this field have been mainly concerned with the ability of animals to withstand transfer from fresh to salt water and vice versa, and with the physiological effect upon them of such transfer. Loeb and his co-workers, however, have investigated the influence of many different salts, with especial reference to their osmotic effects and the counter-action of one salt upon another,[1] while Wells (1915a) and Garrey (1916) have studied the resistance of several species of fresh water fish to solutions of various salts.

The influence of alkalinity and acidity upon fish have also received some consideration, especially in reference to their reactions.

The results of these experiments are widely divergent. In some cases direct transfer of fresh water organisms to sea water has been possible or vice versa, while in others such attempts have met with failure. The reasons for such divergence are not far to seek.

In the first place the standards of experimentation have been widely different. There has been no set time which an animal must live in order to be said to have survived the transfer, experimenters in most

[1] See especially Loeb (1912 a, pp. 169–192).

instances contenting themselves with stating that in one case the survival period has been any given number of days, while in other cases death has ensued in a few minutes or hours. Furthermore there is no agreement as to what per cent of survivals shall constitute success and what failure.

Second, it is obvious that no two species and no two individuals are alike in their ability to withstand environmental changes; hence experiments conducted on different species and on small numbers of animals are bound to diverge widely in their results.

Third, the physiological condition of the individual varies at different times, dependent upon various factors, many of which are obscure, but some of which, such as food, temperature and the like are sufficiently obvious. A group of fishes, for example, taken from acid or neutral water, would be expected to respond differently to an alkaline medium than would those already acclimated to such a medium.

Fourth, the apparatus and number of animals used in making the tests have differed with the means and the purposes of the individual investigator. In general the numbers employed have been small, and insufficient attention paid to such matters as feeding, aeration and the like.

Fifth, acclimatization may play some part in determining survival, but in most cases this question has been neglected.

Space need not be devoted here to reviewing the previous work in this field, as this has already been done by Sumner (1906), Scott (1913) and Garrey (1916).

In connection with an investigation of Devils Lake, North Dakota, conducted for several years by the State Biological Station, the writer together with Dr. C. E. King, formerly of the University of North Dakota, has made a study of the resistance of fish to solutions of the salts commonly found in alkaline waters, in an effort to determine the degree of concentration which fish will withstand; and to determine whether death is due to osmotic pressure, chemical toxicity or other factors.

Devils Lake is one of many lakes in the western United States, which through lack of inlet and excess of evaporation over precipitation is gradually drying up and steadily increasing in salt content and alkalinity. According to early settlers in the region it formerly swarmed with pickerel and, until recently, the stickle-back (Eucalia inconstans) was very abundant there. Lord (1884) reported a few "shiners" and Pope (1908) stated that the minnow (Pimephales promelas) was

abundant in 1907. No minnows have ever been found by me in the lake, and the stickle-backs, which until 1915 were fairly common, are now less numerous than formerly.

Beginning with the experiments of Pope in 1907 and Brannon in 1909 numerous attempts have been made to stock Devils Lake with fish which, prior to 1915, met with a fair degree of success,[2] the experimental fish, mainly yellow perch, living in lake water for many weeks, while in the spring of 1914 several specimens were seined in the lake. Coincident, however, with the constantly increasing concentration of the lake water, the fish, since 1915, have lived only for brief intervals after transfer to full strength lake water.

In addition to the earlier experiments with fish, some on the transfer of Protozoa performed in 1917 by Prof. C. H. Edmondson[3] are of interest. In these experiments various species of Protozoa in infusions from a fresh water pond near Devils Lake were suddenly transferred through several changes into pure lake water and vice versa with the following results:

Three species from the fresh water pond (Paramecium sp., Stylonichia sp. and Metopus sp.) transferred to lake water all died in from 10 to 32 minutes. Prior to death there was abnormal behavior, gradual cessation of movement and more or less distortion. Similarly three other species (Uroleptus sp., Euplotes patella and Uronema marinum) were transferred from lake to fresh water. With the first two there was marked distortion and swelling, evidently due to imbibition of water with accompanying cessation of movement. Gradually, however, in most cases, the organisms resumed their normal form and activity, becoming apparently fully adjusted to their new environment. Somewhat similar results are recorded by Sumner (1906) in the transfer of marine fish to fresh water, viz., an initial gain followed by loss in weight. With Uronema marinum, an inhabitant of both fresh and salt water, there were no apparent effects of the transfer.

The species of fish employed in the following experiments were chiefly yellow perch, although several species of sunfish (Lepomis gibbosus and incisor), catfish (Ameiurus nebulosus), suckers (Catostomus commersonii), minnows (Pimephales notatus), shiners (Notropis cornutus, and hudsonius), sand rollers (Percopsis omisco maycus) and darters (Percina caprodes zebra) were also used.

[2] See Pope (1908) and Brannon (1911, 1913).

[3] See Edmondson (1920).

Previous experimenters have usually employed solutions in distilled water, which may in itself be injurious to fish, but is most likely so by reason of the *lack* of substances present in natural waters.

In the experiments here described the attempt has been made to determine the killing strength of solutions of salts, most of which occur commonly in nature, in waters *natural* to the fish. The solutions have also been brought gradually up to the killing strength, thereby affording considerable opportunity for acclimatization of the fish. In most of the experiments the fish have been fed and the aquaria have been larger than those employed by previous experimenters, thereby affording the fish a more natural environment.

The experiments were conducted in closed systems comprising a wooden supply tank of 500 gallons capacity, and aquaria holding from 140 to 190 gallons each, a sand filter and a catch basin of concrete. By this arrangement it was possible to use the same water repeatedly for several weeks, during the course of any experiment, thereby obviating the prohibitive expense which would have been entailed had it been necessary to add fresh chemicals in large amounts (50 to 100 lbs. in some cases) to the water daily. Usually two experiments were conducted simultaneously, with a single control for both.

In addition to those conducted in the tanks a few experiments were performed in small glass aquaria employing but a few specimens of fish in each case. The water in these experiments was aerated by spraying it into the aquaria through fine glass nozzles. In the tank aquaria this provision was scarcely necessary, the water receiving abundant aeration in its passage through the sand filters and in turning it over from the catch basins to the supply tanks; but in the glass aquaria considerable difficulty was experienced in aeration due to clogging of the nozzles, so that in some cases the oxygen supply fell dangerously low, with occasionally fatal results.

In general the fish were held for several days, in some cases weeks, in well or cistern water before being placed in the experimental aquaria. The salts were then added in fractional amounts at 4-day intervals, and after the water in the aquaria had been turned over once or twice to insure thorough mixing of the solution, the concentration of the latter was read.

In experiments 1, 5, 6 and 8, these readings were approximate only; in the others exact methods were employed. In one set of experiments, because of the approach of winter and the consequent necessity of bringing the year's work to a close, the interval was shortened to 2 days,

while occasionally it was somewhat lengthened.[4] The time of death of each fish was recorded, the experiment being continued until most or all were dead. In many cases the appearance of the fish after death was noted, while in those experiments performed in the glass aquaria where it was possible to see them, their behavior prior to death was noted in many instances. In at least one experiment with each salt the osmotic pressure of the solution was taken at or near the end point.

Records of temperature, free CO_2 and of carbonate and bicarbonate alkalinity were taken,[5] and, in the glass aquaria, of the oxygen as well. In the tank aquaria, which were adequately aerated, the latter reading was soon found to be unnecessary and was consequently but rarely made.

Commercial salts were used in the experiments since the cost of the purified products would have entailed too great expense. Analyses made by Drs. G. A. Abbott and C. E. King of the University of North Dakota showed negligible amounts of contamination. Complete analyses were not made as this would have been a needless refinement in view of the presence of various impurities in the water employed for the experiments.

The latter was obtained from two sources, cisterns and a deep well. Analyses of both kinds of water are given in table 1.

TABLE 1

Analyses of well and cistern water used for experiments

Analyses (except as noted) made by Dr. C. E. King, the former on sample from well, and the latter on sample taken from glass aquarium containing fish (8/28/1919). The character of the cistern water naturally varied somewhat from time to time, but this analysis gives a sufficiently clear idea of its character.

	WELL	CISTERN	
CO_3	0	14*	
HCO_3	764	54	16.0*
Cl	105	10	6.5*
SO_4	608	192	
Mg	49	8	
Ca	180	9.2	
Na	403	48	
K	54	1.5	
SiO_2	32		
$Fe_2O_3+Al_2O_3$	15		
Total solids	1680		

* Analysis by Young on sample from cistern 9/5/19.

[4] In some experiments to test the resistance of fish to alkalinity, to be recorded later, the time interval was much less.

[5] Except as noted in the individual experiments.

The influence of such impurities as were present in both waters was eliminated by the use of the same kind of water in the control as in the experimental aquaria. Considerably greater difficulty during the summer was experienced with the death of fish in the cistern than in the well water. This was in part due to the development of fungus, but in many cases fish died in the cistern water for no apparent reason. With the approach of colder weather this difficulty disappeared. The elimination of the weaker fish during the earlier part of the season as well as the change in temperature may in large measure account for this difference.

In the tank aquaria the fish were fed regularly in most experiments and usually ate greedily until the end point was nearly reached.[6] In the glass aquaria feeding was attempted but was usually unsuccessful.

Relative to these experiments, the question arises whether the fish would live for any considerable period at a concentration a little lower than the killing strength, or whether they might not die at such a concentration if the latter were given sufficient time to act upon them. While a definite answer to this question is impossible under the conditions of the experiments, a suggestion is given by two experiments in which a few fish were held for several weeks at a concentration about 75 per cent of that at which they died.[7]

The experiments may be best presented as a table, giving the data and results of each experiment in summary form. The determinations of osmotic pressure at the end point in most of the experiments were made later on solutions of approximately the killing strength. Where this is the case, the latter has been given in parenthesis. The slight inaccuracy involved is not significant in view of the great differences in resistance of individual fish.

When two readings are given in one experiment, these indicate approximately the limits between which the fish died. The well water has an osmotic pressure of 1.1 atmospheres while that of the cistern water is only about 0.1 atmosphere, hence the same strength of solution of any given salt has a somewhat greater pressure when dissolved in well water

[6] In the alkalinity experiments the fish often lived for a considerable time after ceasing to eat.

[7] See experiment 5.

than when it is dissolved in cistern water. Not all of this difference is effective, however, since some of it is due to sulphates and chlorides, and hence is included in the effect of the experimental salt.

In addition to the experiments described above a few were performed to test the resistance of fish to sodium carbonate alkalinity. Wells (1915 b) claims that "fresh water fishes cannot live normally in water that is alkaline" to phenolphthalein, the fatal degree of alkalinity in his experiments being about 50 ppm. of Na_2CO_3. His experiments were very limited, however, so far as the resistance of the fish was concerned. The fact that Wells used distilled water in making his solutions may possibly explain his results, since distilled water itself is generally injurious to fish.

The large concrete aquaria and cistern water were used in these experiments, and the data are given in tables 3 and 4.

The alkalinity in the control aquarium for experiment 14 was CO_3, 15 ppm., HCO_3, 9 ppm. on 8/7; CO_2 trace, HCO_3, 700 ppm. on 9/2.

There are two points of particular interest in this experiment; first, the comparatively rapid death rate of the fish in the earlier part, and second, the extreme resistance of a few individuals. The former point is probably explicable by the fact that the fish had only recently been brought to the laboratory and may not yet have recovered from the shock of their journey. This explanation is borne out by the further fact that there was a considerable mortality in the control at this time. The recovery of the fish from a "distressed" or "dying" condition, as happened in several instances after removal to the control, proves the alkalinity to have had an injurious effect upon them, however, which effect was probably greater owing to the influence of their recent journey. It is further worthy of note that, in the case of the resistant fish, the alkalinity made itself felt long before the killing point was reached, as is proven by the loss of appetite of the fish; and similar results have been obtained in other experiments.

In the two following experiments (15, 16) controls were run under conditions similar (except alkalinity) to those of the experiments, but alkalinity readings were not made.

In connection with these experiments, it is worth while to inquire as to the alkalinity of natural waters inhabited by fish and the limits which they can withstand, judged by their distribution in these waters. Here, of course, it is impossible to reduce the problem to one of alkalinity alone, since so many complicating factors enter; nevertheless the comparison is not without interest.

TABLE

EXPERIMENT	AQUARIUM	WATER	SALT	FISH	BEGUN	ENDED	TEMPERATURE	O_2 CC. PR. L	CO_2 PPM.	CO_2 PPM.
1	glass	cistern	Na_2SO_4	2 Perca flavescens	8/14/19	8/21/19	18°–19°	0.8–3.0	Tr.–10	0
2	glass	cistern	Na_2SO_4	3 Perca flavescens	8/10/20	8/28/20	20°			0
3	tank	well	Na_2SO_4	29 Perca flavescens	9/ 8/19	10/12/19	17°–7.5°		6–0	Tr.–60[8]
4	tank	well	Na_2SO_4	12 Perca flavescens 2 Ameiurus nebulosus	9/ 1/20	10/17/20	20°–10°	7.0 approx. 10/15	0–9	0 20,(10/17)
5	tank	cistern	Na_2SO_4	2 Perca flavescens 2 Percina caprodes zebra 1 Catostomus commersonii 4 Ameiurus nebulosus 2 Ameiurus nebulosus added 9/22 2 Lepomis gibbosus	9/ 4/21	11/12/21	20°–0°		Tr. 9/3 Soon disappeared	0–22
6	tank	well	$MgSO_4$	36 Perca flavescens	8/ 6/19	10/23/19	20°–2°	5.0 average	10–0	0–110[8]
7	tank	cistern	$MgSO_4$	24 Perca flavescens	10/26/19	11/ 9/19	0.5°–0°		0	25–45
8	glass	cistern	NaCl	2 Perca flavescens	9/ 2/19	9/12/19	15°–16°	0.8–3.2	Tr.–12	10–0
9	tank	well	NaCl	35 Perca flavescens	8/ 7/19	9/ 4/19	20.5°–15.5°	5.0 (average)	Tr.–0	0–22
10	tank	cistern	NaCl	14 Perca flavescens 1 Ameiurus nebulosus	9/ 1/20	10/ 3/20	16.5°–9.5°	7.0 (approx.) 10/15	0.7	0
11	tank	cistern	$CaCl_2$	24 Perca flavescens	10/29/19	11/ 8/19	1°–(–)0.5°		0	20–3
12	tank	well	$CaCl_2$	18 Perca flavescens 2 Ameiurus nebulosus	7/26/20	8/ 6/20	18°–20°			0
13	tank	well	KCl	5 Perca flavescens 1 Ameiurus nebulosus	8/19/20	8/21/20	20°–19.5°		0	65–45

[8] In both experiments 3 and 6 CO_3 ran rather high (up to 60 and 110 ppm. respectively). It is possible that these alkalinities may have hastened the death of the fish. This is unlikely, however, since they occurred in both experiments independently of their death and since in the experiments described below fish withstood much higher alkalinities than these.

[9] In
held for
18475.

CO_2 PPM.	HCO_3 PPM.	SURVIVORS	END POINT			NOTES
			Concentration ppm.	Osmotic pressure, atmospheres	Dissociation, per cent	
0	50–60	0	13400	5.1	73.6	O_2 averaged higher and HCO_3 somewhat lower in control than in experiment
0	65–100	1	14700	5.7	73.6	HCO_3 somewhat lower in control than in experiment. 8/20, 1 fish dead in control, cause?
Tr.–60[8]	365–260	1	15100–18600	(14950) 6.6 (18500) 7.6	73.6	No temperature or alkalinity readings were made after 10/9. Same control used for experiments 3, 6 and 9. In all three the death rate was higher in the control than in the experiments until the end points were reached, when the fish died rapidly in the latter, while living well in the former
0 20,(10/17)	55–140	0	18300	7.4	73.6	1 perch died in control, 9/5. Most deaths occurred before the indicated end point was reached
0–22	70–116	2 Lepomis gibbosus	18400		73.6	Note survival of 1 sucker, 2 perch and 2 sunfish for more than a month at a concentration of 14900, while 3 of them (the sucker and perch) died at 18400[9]
0–110[8]	365–245	0	24500–27500	(24200) 5.6 (27300) 6.6	22.3	Prior to 8/17 several deaths occurred, apparently caused by some infection, possibly fungus. After cleaning and refilling the system no further deaths occurred, with one exception, until the end point was reached
25–45	35–55	0	20900–28400	(20700) 4.8 (28300) 6.4	22.3	CO_2 in control, 12–2 ppm.
10–0	22–32	0	15450	(15350) 11.2	85.0	
0–22	310–210	0	13500	(13350) 10.5	85.0	1 fish died prior to 8/11. System refilled on 8/17 and no further death occurred until the end point was reached
0	60–105	0	9100–17550	(9200) 6.8 (17550) 12.8	85.0	1 perch died in control, 9/5
20–3	22–45	0	9500–13500	(13300) 7.3	81.5	
0	360–50	6 Perca flavescens 1 Ameiurus nebulosus	11100	6.6	81.5	CO_2 in control, 25–65, HCO_3 360–240 ppm.
65–45	240–215	1 Perca flavescens 1 Ameiurus nebulosus	1360	2.0	91.8	CO_2 in control, 25–65, HCO_3 360–240 ppm. Minimum lethal amount of KCl not determined

[9] In another experiment, details of which may be omitted, a sunfish (gibbosus) was held for about 20 days in an NaCl solution of 14300 + ppm., dying at a concentration of 18475.

TABLE 3

EXPERIMENT	DATE	FISH	TEMPERATURE	CO_2 PPM	HCO_3 PPM	
14	8/7/21	10 Perca flavescens 5 Percina caprodes zebra 1 Lepomis incisor 1 Lepomis gibbosus 1 Notropis cornutus 1 Pimephales notatus	15°–21°	18	17	3 darters dead in control
	8/8			75		1 darter dead in experiment 1 perch dead in experiment 1 perch dying in experiment 1 darter losing balance in experiment 1 darter dying in experiment Both latter transferred to control, where they revived, 1 of them, however, was dying on 8/13, while the other appeared healthy on 8/18, when last observed
	8/9			95		1 perch losing balance, and 1 dying, transferred to control, where both revived. 1 shiner and 1 darter dead
	8/10			100		1 darter dying
	8/11			120		1 perch distressed
	8/13			135		1 perch dead
	8/14			150		1 perch dead 1 small fish losing balance
	8/15			160		1 fish at surface "dashing wildly about"
	8/17			185		Fish not eating well in experiment, but eat readily in control
	8/22			300		1 perch distressed
	8/24			350	140	1 perch dead Pimephales dead
	8/26			385	145	L. incisor dead
	8/28			430		1 perch distressed
	9/1			600	200	1 perch dead L. gibbosus dead

Few, if any, data are available as to the distribution of fish in relation to the chemical nature of their environment. In North Dakota studies of a number of lakes in an effort to determine their suitability for fish culture showed that the highest alkalinity in any lake supporting larger

TABLE 4

EXPERIMENT	DATE	FISH	TEMPERATURE	CO_3 PPM.	HCO_3 PPM.	
15	7/12/21	5 Ameiurus nebulosus	20°–21°	35*	45*	
	7/14	1 Percopsis omisco maycus		100		
	7/20			415		Catfish restless, sand roller dead
	7/23			470		2 catfish losing balance, others inactive
	7/24			540	185	1 catfish dying
						4 catfish inactive, transferred to control, 3 recovered, 1 dead, 7/25
16	7/26	3 Ameiurus nebulosus	17°–20°	0	400	
	7/27					
	6:00 a.m.			130		
	8:00 a.m.	1 Percopsis omisco maycus				
		1 Notropis hudsonius				
	2:00 p.m.			310		Shiner dead
	8/1			440		1 catfish losing balance
	8/2					Sand roller losing balance
	4:00 p.m.			240		
	9:30 p.m.					Dead
	8/3			465		1 catfish losing balance
	8/4			560		2 catfish losing balance
	8/5			690	350	2 catfish dying
						1 catfish losing balance, transferred to control, recovered

* Approximate.

fishes (perch, pickerel, etc.) was CO_3 72 ppm. The stickleback (Eucalia inconstans) and minnow (Pimephales promelas), however, occur in water with CO_3 alkalinities running up to over 500 ppm., while the killifish (Fundulus diaphanus) is found in a lake near Streeter, North

Dakota, with an alkalinity of 680 ppm. Devils Lake water has an alkalinity of over 200 ppm. yet fish can live in it for several days, and occasionally even weeks, while it is not many years since they were able to live in it indefinitely, although it had at that time an alkalinity CO_3 of nearly 200 ppm.

It is difficult to make a fair comparison between my own results with CO_3 alkalinity and those of Wells (1915b), since his experiments are so scanty, so far at least as the resistance effect is concerned. While the fish with which he experimented in general preferred slight acidity to slight alkalinity, this reaction in no way proves their inability to live in the latter. While there is wide divergence in my own results in respect to the resistance of fishes to CO_3 alkalinity; nevertheless these results, together with the occurrence of fish in alkaline waters, prove that Wells' conclusions regarding the effect of alkalinity on fresh water fish are entirely too far-reaching.

As to the general cause of death of the fish in Devils Lake water and in the solutions of various salts in the experiments described above, there are two major possibilities; one osmosis and the other toxication. The first is suggested as an explanation by the fact that at the killing strength the osmotic pressure of many of the solutions employed and of the lake water is approximately the same, i.e., about six atmospheres, which is about that of the blood of many species of fresh water fish as shown by Garrey (1915). Considerable variations do of course exist, but these are for the most part no greater than those in the resistance of individual fish. The fact that the end point in many of the solutions occurs at or near the osmotic concentration of the blood of the fish suggest that the gill membranes are impermeable on the one hand to the salts in solution and on the other to the water of the blood, but that when the critical point is reached this impermeability is overcome, permitting interchange of substances between the body of the fish and the surrounding medium, disturbing the chemical equilibrium of the body fluids and resulting in death. Unfortunately for such an hypothesis, however, Sumner has shown that several species of marine fish can withstand transfer to water of low salinity (S. G. 1.002–1.001) without material permeation or apparent ill effects. Water of this concentration has a much lower osmotic pressure than that of the blood of the experimental fish, which averages about nine atmospheres. Sumner has also shown that in many cases changes of weight occur, (indicating permeability) without apparent ill effects upon the fish.

In the case of the KCl experiment (no. 10), however, the low end point indicates clearly a toxic effect, when compared with the end points of the other salts employed, and further the end points in the NaCl solutions are in general much higher (about 11 atmospheres) than in the others. Undoubtedly osmotic pressure is a factor of much importance in determining the results, but that it is the most important one is open to question.

There is no constant relation between the degree of dissociation of the salts employed and their killing strength. It is true that for the sulphates, especially that of magnesium, which have a relatively low degree, the concentrations at the end point are much higher than are those of the chlorides, whose degree is relatively high, but between the chlorides themselves no such relation exists. Thus $CaCl_2$ is more toxic than NaCl although the dissociation degree of the latter is higher than that of the former; while KCl, which at the end point concentration, has only 7 per cent greater dissociation than NaCl, is at least ten times (1000 per cent) more toxic.

My results agree with those of Garrey (1916) in regard to the relative toxicity of K, Ca and Na in chloride solutions. I have not tried $MgCl_2$, but in sulphate solution Mg is less injurious than Na, which is doubtless due to the much higher dissociation percentage of the latter.

Occasional reference has already been made to the appearance and behavior of the fish at, and prior to death. One of the most marked symptoms was the irregularity of respiration. Normally regular, prior to death the respiratory movements were greatly reduced in number and increased in force, coming in spasmodic gulps of two to four, with intervening periods of several seconds' rest. Prior to death also the fish were usually restless and easily excited, any slight stimulus causing them to dash excitedly about the aquarium. This restlessness was frequently spontaneous, occasionally leading to suicide on the part of the fish, by leaping out of the aquarium onto the floor. With fish which have been living for some time in lake water a slight shock may cause temporary loss of balance. The influence of this water evidently renders the fish peculiarly sensitive to any slight injury. Loss of equilibrium was also a marked feature, the fish at first swimming on their sides, then on their backs, and righting themselves only with a distinct effort.

The fish frequently, though not universally, died with distended jaws and gill covers, while congestion of the face, opercula and fin bases was a common feature. Occasionally the eyes became opaque, though whether this opacity was in the lens or cornea or both was not deter-

mined. In Na_2CO_3 alkalinities of about 800 ppm. catfish became covered with a thick coat of slime, and in some instances there was marked inflammation and abrasion of skin around anus and on ventral fin; while in KCl solutions there was rapid disintegration of the fish which died. Wells (1915a, p. 273) has pointed out the disintegrating effect of calcium solutions on fish, attributing the reduction of the tail of fish in certain British waters to an excess of this salt in the water. What the explanation is I do not know, but do not consider the effect specific to calcium.

Similar observations have been made by several previous writers. Bert (1871) describes the agitation, irregular respiration, congestion of gills and opacity of lens and cornea in fresh water fish transferred directly to sea water; while loss of equilibrium and sensitivity to shock are described by Siedlecki (1903) in the case of fish placed in solutions of glycerine. Wells (1913) introduced several species of fish into varying combinations of low oxygen and high CO_2 and noted similar loss of equilibrium, irregularity of respiration and excited behavior of the fish in the fatal combinations.

Opinions differ as to the relative resistance of small and large fish, Bert (1883) maintaining that the larger the individual the greater its resistance, while Siedlecki found that specimens of medium size, well-nourished and vigorous, could resist better than larger ones which were soon enfeebled in captivity. Sumner found no evidence of "selective mortality in relation to size among fishes dying from the effects of fresh water," but "such a selective mortality was very obvious in the case of death from asphyxiation." In my own experiments with fish of medium size (yellow perch 3 to 8 inches, sunfish 2 to 3 inches, catfish 3 to 10 inches), I have observed no relation between mortality and size.

As to the immediate cause of these effects we have as yet no certain knowledge. Some authors, Sumner (1906, 1907), Scott, (1908, 1913) and others have pointed out the change in weight which occurs when fish are suddenly immersed in solutions which are either hyper- or hypotonic to their own blood. Such a result is clearly osmotic. Loeb and Wasteneys (1915), while admitting the osmotic effect, believe that death is caused by the toxic action of a non-balanced solution on the tissues, or by its action in inhibiting respiration through a nervous reflex. Loeb (1912b) attributes the death of Fundulus in sugar solutions to the formation of acid, but in concentrations greater than M/4 these are in themselves harmful, apart from any action of acid.

The appearance and behavior of the fish prior to death strongly suggest a suffocation effect, associated with, or more likely the cause of arrested circulation and disturbance of the nervous system, as shown by the loss of balance. But the immediate cause of these symptoms and of death itself remains unknown. Unquestionably osmosis is primarily responsible, but is it wholly so? Are changes in the gill membranes themselves due to the action of dissolved ions, and interfering with normal respiratory changes responsible for death? Or is osmosis merely one link in the chain of events, whereby toxic substances gain entrance to the body, there to initiate a series of reactions finally ending in death? And are these reactions primarily between the toxic agent and the nervous system, inducing through the improper action of the latter a series of pathological changes throughout the body; or is the action of the former upon the general tissues a direct one, and the nervous effects (irregularity of respiration, excitability, loss of balance) secondary? These are questions for the future to decide.

While these experiments have contributed but little to our knowledge of the mechanism of the death of fish in hypertonic solutions, they do indicate that the lethal action of most of our alkaline waters is a composite one, and not due to the single action of any particular salt, since the concentration of any salt in natural waters seldom equals the lethal concentration of such salt in the experiments. They also give at least an approximate standard for estimating the fitness of any water for fish culture, and one means of interpreting the distribution of fishes in our inland waters.

They show further that it is not safe to rely upon a single factor, such, for example, as alkalinity, in estimating the suitability of any water for fish, but that all should be considered, since one factor may counteract another.

The individual resistance of different fish renders it obviously impossible to establish any exact standard; but for the species employed, and these are probably fairly representative ones, any water with an osmotic pressure greater than 6 atmospheres is of doubtful suitability for the introduction of fish.

Note: Since the above was written I have received a paper by Professor Huntsman, (Huntsman, A. G., 1922. The Quill Lakes of Saskatchewan and their Fishery Possibilities. Contributions to Canadian Biology, being Studies from the Biological Stations of Canada, No. 9), in which he describes the occurrence of several species of fish, (Pygosteus pungitius, Catostomus commersonii, Esox lucius? and Perca flavescens) in Big and Little Quill Lakes, the former of which has a total solid content of 16550 and OH alkalinity of 134 ppm. and the latter a

total solid content of about 11000 and OH alkalinity of 122 ppm. Only the sticklebacks and suckers were observed by Huntsman, the others being reported by local fishermen. But a single specimen of the perch was reported, "said to have been taken in Big Quill Lake . . . . . about 1918." Both of these lakes are connected by streams with other lakes, at least during high water in spring, and it is from the latter that the former are believed to have been stocked.

Little Quill Lake is a little below the critical point in salt content for most fresh water fish as determined by my experiments, while Big Quill is a little above that point. As regards the sticklebacks there is no contradiction in our results, while my experience with suckers has been rather limited. The number of dead fish found on shore in the spring may mean that the fish do not live long in these lakes, the supply being maintained from their tributaries. It is possible also that the fish live mainly near the mouths of the streams, where the water should be much fresher than elsewhere.

BIBLIOGRAPHY

BRANNON, M. A. 1911 1st and 2nd annual repts., N. D. Biol. Station. Public document no. 33.

BRANNON, M. A. 1913 3rd and 4th annual repts. Public document no. 35.

BERT, P. 1871 Compt. rend. d. l'Acad. d. Sci. lxxiii, 382, 464.

BERT, P. 1883 Compt. rend. d. l'Acad. d. Sci. xcvii, 133.

EDMONDSON, C. H. 1920 Trans. Amer. Mic. Soc., xxxix, 167.

GARREY, W. E. 1915 Biol. Bull., xxviii, 77.

GARREY, W. E. 1916 This Journal xxxix, 313.

LOEB, J. 1912a The mechanistic conception of life. University of Chicago.

LOEB, J. 1912b Journ. Biol. Chem., xi, 415.

LOEB, J. AND H. WASTENEYS. 1915 Ibid., xxi, 223.

LORD, H. W. 1884 Bull. U. S. F. C., iv, 351.

POPE, T. E. B. 1908 U. S. Bur. Fish., Doc. 634.

SCOTT, G. G. 1908 Bull. U. S. Bur. Fish., xxviii, 1145.

SCOTT, G. G. 1913 Biol. Bull., xxv, 121.

SIEDLECKI, M. 1903 Compt rend. d. l'Acad. d. Sci. cxxxvii, 469.

SUMNER, F. B. 1906 Bull. U. S. Bur. Fish., xxv, 53.

SUMNER, F. B. 1907 This Journal, xix, 61.

WELLS, M. M. 1913 Biol. Bull., xxv, 323.

WELLS, M. M. 1915a Journ. Exper. Zoöl., xix, 243.

WELLS, M. M. 1915b Biol. Bull., xxix, 221.